COMPARISON OF CLASSICAL RICKETS WITH OSSEOUS CHANGES IN BABY PIGS FED A RATION DEFICIENT IN D VITAMINS

by

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INTRODUCTION

Rickets is a well described disease occurring in infancy and early childhood (1, 11, 64, 101, 112). Osteochondral lesions similar to rickets have been frequently reported in many domesticated farm and laboratory mammals including the pig (25, 76, 91, 102, 104, 126, 127, 157).

During the period of 1958 to 1960, more than forty per cent of the pigs examined in the Iowa Veterinary Medical Diagnostic Laboratory have been observed to have osteodystrophy. Many of these pigs have had pliable ribs, enlarged costochondral junctions and distorted epiphyseal lines. These cases have been frequently diagnosed as "rickets" on the basis of the clinical history and gross appearance, but have not been confirmed by histopathological examinations.

In 1957, Aaron Groth, Jr. (48, 49) surveyed 46 Iowa pigs which had pliable ribs and enlarged costochondral junctions for microscopic lesions of rickets. He was unable to demonstrate any histologic evidence of rickets and suggested the lesions observed resulted from interference with osteogenesis by infectious viral agents, such as hog cholera and swine influenza, which were possibly associated with diets deficient in calcium and A vitamins.

In 1959, because of the primary role of D vitamins in the

development of classical rickets in the infant (1, 32, 44, 55, 112, 166), an experiment was proposed to study the effect of a ration deficient in D vitamins on bone development in newborn baby pigs. This thesis is the result of that experiment.

REVIEW OF LITERATURE

Classical Rickets (Infantile)

Classical rickets refers to a specific nutritional disease in the human being occurring during infancy and early childhood (1, 11, 23, 102, 162). It occurs most frequently during the period from four months to two years of age (1, 64, 67, 69, 114). It is characterized by defective nutrition of the entire body with greatest expression in the skeletal system (29, 162). Rickets is essentially a disturbance of calcium and phosphorus metabolism resulting from a primary deficiency of D vitamins (1, 32, 44, 55, 112, 162, 166). This vitamin deficiency may also be accompanied by secondary dietary calcium and phosphorus imbalances (1, 64).

The early symptoms of rickets are restlessness, fever, profuse sweating and a general sensitiveness of the body when touched (11, 67, 114, 162). Later, the disease manifests the symptoms and signs associated with the characteristic skeletal lesions.

These skeletal changes are the result of defective mineralization of the bone and cartilage (6, 11, 23, 112, 115). The bones are soft and pliable (1, 11). Mechanical stresses resulting from the weight of the body and muscular action on these soft bones produce corresponding distortion such as

bending of the femur laterally, bending of the tibia anteriorly and curving of the spine posteriorly or laterally (11, 64). The sternum is pushed forward (11) by abdominal pressure and the costochondral junctions of the ribs are pulled inward by the muscles of inspiration, principally the diaphragm (64). This distortion results in the presence of a vertical groove in each side of the thorax (11, 64). Epiphyseal proliferation gives rise to a series of nodular enlargements at the costochondral junctions and nodular swellings at the wrists, knees and ankles. The cranial bones are also distorted with thickening of the spongy bone in the frontal and parietal regions of the skull causing the skull to become box-like and thinning of the bone in the back of the skull (11, 64, 67). The closure of the fontanels is delayed (102, 162, 61).

In addition, clinical examination may reveal anemia, splenomegaly, lymphoid hyperplasia, flaccid musculature and poor development of the teeth, (11, 64).

Examination of the blood reveals a low level of inorganic phosphorus in the serum which early in the disease is usually accompanied with normal or slightly decreased levels of calcium in the serum (11, 50, 55). Later in the disease, serum levels of calcium may also decrease as the result of parathyroid exhaustion of the calcium stored in the bones (50). The amount of alkaline phosphatase in the serum is greatly in-

creased as the result of stimulated osteoblastic activity (50).

In radiographs, the metaphysis appears indistinct, frayed or characteristically concave, "saucer" or "cup-shaped" (20, 64, 143). The epiphyses are thicker and wider than normal (1, 11, 115). The shafts of the bones are less dense and are deformed as a result of muscle and tendon tension (11). Sometimes incomplete, green-stick, fractures may be seen (1, 112).

Macroscopic lesions

Post mortem examination reveals, in addition to the previously described clinical alterations, deformities of the vertebrae and pelvic bones and medial projection of the costochondral junctions (11, 116). The medial projection at the costochondral junctions sometimes becomes so great that the lungs may become compressed and bear impression of the ribs (116). Rarely, the thorax may collapse resulting in death by asphyxiation (116).

The bones are soft and pliable (1, 11, 64, 112). The epiphyses are cut easily with a knife (1, 11, 64, 112). If the long bones are sectioned longitudinally, the epiphyseal plate of cartilage is broader than normal (62). The cartilage is bluish-red, enlarged and soft. The linear zone of mineralization is irregular and often indicated only as scattered whitish points. The marrow is hyperemic and the vascular medullary spaces have penetrated the soft cartilage beyond the

line of preliminary mineralization. The medullary spaces are irregular. Between the cartilage and hard bone at the metaphysis, there often lies a soft, flexible and elastic layer of osteoid tissue, which may vary in width, from a few millimeters to one or two centimeters (62). The epiphyses are enlarged botn in width and in breadth (1, 11, 64, 115). If the periosteal and endosteal tissue contains extensive amounts of osteoid tissue, the shaft of the bone is soft, pinkish in color and easily cut (62).

Microscopic lesions

The basic tissue alteration is a failure of mineralization of the cartilaginous matrix substance and the osteoid tissue of the trabeculae (1, 11, 112, 115). These lesions are evidenced by an absence of deep bluish-red staining granular material in the osteochondral tissues when standard techniques are used for decalcification, for sectioning and for staining with hematoxylin and eosin solutions (1, 11, 115). The zone of cartilage proliferation is enlarged both in width and breadth (1, 11, 115). This zone may become more than ten times its normal width (115). Near the metaphyses, the cartilage cells lose tneir normal straight arrangement in columns, become hypertrophic and send prolongations or "tongues" of cartilage into the metaphysis, which gives the line of preliminary mineralization the irregularity so characteristic of

the gross appearance. The normal columnar arrangement of the cartilage cells of the zone is completely absent or is evident only at its base. These cells have various sizes and shapes. The epiphyseal plate of cartilage is irregularly penetrated by branching blood vessels from the metaphysis. The branching of these vessels is in an arboreal pattern which gives them a bush-like appearance (115). The vascular channels are accompanied by connective tissue and osteoblasts and are often surrounded by osteoid tissue. Vessels in increased numbers extend into the cartilage from the perichondrium.

At the metaphysis, the spongiosa is greatly altered (115). The trabeculae have lost their orderly arrangement and have become irregular in shape and size. They are principally composed of osteoid tissue. These osteoid trabeculae may contain a central core of bone or entrapped cartilage cells. Osteoid tissue is found in excess not only at the metaphysis but also along the perichondrium and the cortex.

Osteoid tissue is produced by osteoblasts and metaplasia of cartilage (21, 22, 115). Osteoid tissue may be either homogeneous or fibrillar (62). It sometimes has a lamellated arrangement. Osteocytes are present in an irregular pattern. In severe cases, the osteoid tissue may be produced in such a quantity that the spaces in the existing bone, as well as the medullary cavity, are filled (62). Varying degree of fibrosis

may be present in the marrow tissues (1, 64).

Rickets is fundamentally identical to two other nutritional diseases of bone in man, adult rickets (osteomalacia) and juvenile rickets (29, 64, 143). However, these diseases are very rare, occur under unusual circumstances and differ in several respects from classical, infantile, rickets. Therefore, they are classified as separate and distinct diseases. Both juvenile and adult rickets are the result of a primary deficiency of D vitamins (143). They are frequently found to be associated with secondary dietary imbalances of calcium and phosphorus (64). Their basic histologic lesion is an excess of osteoid tissue resulting from defective ossification.

Juvenile Rickets (Late Rickets)

Juvenile rickets (64, 143) occurs in man between the sixth and the eighteenth year of life as a result of deficiencies in D vitamins. The symptoms are uniform and have been well-described by several authors. The onset is characterized by pain on standing or walking, rapid fatigue and pains in the back, hips and legs. The epiphyses of the tibia, radius and ulna become tender and swollen. The gait becomes uncertain and waddling. Skeletal deformities then develop with enlargement of the epiphyses, enlargement of the costochondral junctions, bowing of the legs, bending of the arms and fore-arms and curvature of the spine. The bone deformities however, are usually

less prominent than those of infantile rickets. Tetany is a frequent symptom. Growth is retarded. The spleen and liver are normal in appearance. There is no anemia. There may be interference with development of the teeth.

Juvenile rickets (64) is reported with a much greater frequency in male than in female adolescents. According to Fromme as quoted by Hess (43) the ratio of juvenile rickets in males to females is 15 to 1. This is the result of work in occupations requiring carrying of heavy burdens or walking for prolonged periods of time. The stress and strain of work and the weight of the body on the soft bones leads to easily detectable skeletal deformities. It is believed by some authors that the ratio expressed by Fromme may have been misleading because many females may have been affected by a latent form of juvenile rickets but were not working and failed to develop clinical symptoms with detectable skeletal lesions. It is suggested by others, that the females may have had greater exposure to sunlight and therefore did not develop the disease.

Radiographically, juvenile rickets (143) closely resembles infantile rickets. Fraying of the epiphyses, spreading of the epiphyseal borders and decreased density of the bones are observed. A very important radiographic lesion of juvenile rickets is delayed ossification of the epiphyseal plate. In affected individuals, the epiphyses are often unossified at

the age of twenty years.

Histologically juvenile rickets (64) is characterized by the presence of excessive amounts of osteoid tissue, disturbances in the endochondral bone development and atrophy of the trabeculae. The proliferating epiphyseal plate increases in width and defective mineralization occurs in the provisional zone of mineralization. The metaphysis is very irregular resulting from vascular invasion of the epiphyseal cartilage at various levels and from vesicular cartilage tongues protruding into the diaphyseal region of the metaphysis. These bone changes are usually much less prominent than those seen in infantile rickets.

The bone lesions (143, 64) are similar in some respects to adult rickets (osteomalacia) and similar in other respects to infantile rickets. Because of these similarities to osteomalacia, this disease has also been termed juvenile osteomalacia (64). The similarity of juvenile rickets to both lesions of the infant and of the adult is the result of the difference in the maturity of the skeletal tissues (29, 143). In the bone of the juvenile, the rate of growth is becoming slower and the tissues have begun to assume characteristics of the adult.

Adult Rickets (Osteomalacia)

Osteomalacia, as adult rickets is most frequently termed, is a very rare disease in North America and primarily occurs in the female (11). It is the result of a deficiency of D vitamins in the adult (11, 143). It may be associated with imbalances of dietary calcium and phosphorus (50, 64, 143). In general, the disease usually commences either during puberty or in early adult life. It is often associated with pregnancy and lactation. The close relationsnip of osteomalacia with puberty, pregnancy and lactation suggest hormonal balance may be an important etiologic factor (164).

The onset of osteomalacia is insidious, the first symptom consisting of an indefinite pain in the lower part of the back or in the groin. This pain varies in degree and severity and is a distinctive symptom of the disease. Tetany is frequently observed.

The characteristic lesion of osteomalacia is marked softening of the bone (1, 11, 64, 86). Therefore, skeletal deformities are a characteristic feature of the disease (11, 64, 86). The bones commonly affected are the lumbar vertebrae, the pelvis and the bones of the legs and feet. The sternum and ribs may also be affected. The bones are soft and pliable. The vertebrae may be compressed laterally. The pelvic inlet is distorted and narrowed, making normal delivery at parturition im-

possible. The bones of the legs are markedly bowed. Radiographs of these bones show a faint and lace-like appearance.

Microscopically, the normal bone is almost replaced by osteoid tissue. Broad zones of osteoid tissue surround the trabeculae, the laminae and line the haversian canals. This may be accompanied by atrophy or osteoporosis. Since the epiphyses have closed and become ossified, the only place in the adult skeleton where a cartilage-bone junction persists is the costochondral junction of the ribs (143). Here in the young adult with osteomalacia the same proliferation of the cartilage is found as takes place in the epiphyses of children with rickets. In later adult life, these costochondral junctions also ossify (60).

Rickets defined

Rickets, as defined according to its classic concept, refers specifically to infantile rickets in the human being (11, 23, 162). However, rickets has also been defined by some authors (34) to include all disturbances of bone metabolism in which defective ossification results in excessive osteoid tissue. This broad definition was believed necessary to include in rickets all osteodystrophic diseases of man which histopathologically shows the presence of excessive amounts of osteoid tissue and to apply the results of experimental rickets in animals to bone disturbances in the human being (114).

This is not a satisfactory solution to the problem of defining rickets because the occurrence of osteoid is not associated with deficiencies of D vitamins alone nor imbalances of dietary minerals alone. It must also be emphasized that even though experimental rickets has yielded much valuable information, there is great danger in transference of interpretations of these experiments on bone growth and disease in animals to man (53).

The physiology of bone growth in man differs markedly from that of animals. The skeletal growth of the human is characterized by three stages of rapid growth each of which is succeeded by a period of decreased rate of growth (53). It is during these periods of rapid growth that man is most susceptible to the development of rickets. Man has a specific yet absolute requirement for D vitamins, especially when he is an infant (97). The lack of skeletal development at birth and the length of time required for man to reach maturity are additional factors which lead to his susceptibility to this disease. No experimental animal has been shown to be as susceptible to rickets as man. Only under unusual or experimental conditions can lesions of classical rickets be reproduced in any other genus.

Much of the experimental study of rickets was done during the period of thirty years between 1905 and 1935. Since this time much improvement in research techniques and equipment has

occurred and much additional information is available on the nutritive values of food, information which has invalidated much of the earlier research. Until adequate clinical information is available using standardized modern research techniques and equipment, the application of experimental results from animals to the clinical disease in man must be viewed with definite reservations.

Rickets, broadly defined, is not a single disease. It is a group of diseases which histologically show the common lesion of excessive amounts of osteoid tissue. By this definition, rickets is present both physiologically and pathologically in a number of conditions having fundamentally different etiologic agents. For example, Follis (34), using a broad definition, has defined rickets as "a disease of the growing skeleton which is characterized by a decreased concentration of hydroxyapatite in the organic matrices of cartilage and bone", (that is to say defective mineralization). He outlines those conditions which may exhibit increased amounts of osteoid or defective mineralization as follows: (a) disturbances in the balance of matrix production and deposition of hydroxyapatite, (b) disturbances in intestinal absorption of calcium and phosphorus either singly or concurrently and (c) disturbances resulting in excess excretion of calcium and phosphorus either singly or concurrently.

Conditions which result in disturbances in the balance of matrix production and deposition of hydroxyapatite are: (1) rapid matrix formation, particularly in the premature infant, (2) healing fractures, (3) healing scurvy and (4) healing bone following the removal of a parathyroid tumor.

Conditions which result in disturbances in intestinal absorption of either calcium or phosphorus or both are: (1) a dietary lack of calcium, (2) a dietary lack of phosphorus, (3) a change in the pH of the intestinal contents, (4) formation of insoluble complexes of either calcium or phosphorus or both, (5) steatorrhea, (6) sprue, (7) the presence of poorly utilizable sources of phosphorus such as phytin, (8) the protein content of the diet and (9) a lack of D vitamins. Deficiency of D vitamins may result from: (1) a dietary deficiency, (2) steatorrhea, (3) absence of bile, (4) absence of pancreatic juice and (5) impaired formation in the skin, such as a lack of exposure to the ultraviolet rays of sunlight.

Renal disease caused by either chronic infections or hereditary defects can result in excessive excretion of either calcium or phosphorus or both by the kidneys. Excessive excretion of calcium may occur in pregnancy and lactation.

From the preceding discussion, it has become apparent that the term rickets in medical literature has two distinct meanings. One definition refers to a specific microscopic lesion

of the bone (34). The second definition refers to a specific clinical disease (11).

Rickets is classically defined (23, 162) as a nutritional disease of infancy and early childhood characterized by a disturbance in calcium and phosphorus metabolism which results in defective ossification and is caused by a deficiency of D vitamins. This definition is essentially agreed upon by the majority of medical writers. However, the exact terminology varies greatly depending upon the author and his experience. Often there is disagreement about specific points in the classic definition. Because of these differences, many writers, when discussing this subject, define rickets in their own terms during the course of their articles. This enables the reader to know specifically what the writer has in mind. However, this practice has resulted in hundreds of descriptions and definitions of the disease rickets which vary from each other in innumerable ways. It can usually correctly be assumed if a medical writer does not specify the use of the term rickets, he has reference to classical infantile rickets. This assumption is not true of the so-called "rickets" designation used in veterinary medicine which will be discussed later in this thesis in connection with osteodystrophy in swine.

Experimental Rickets in the Rat

The rat has proven to be a very valuable animal in the study of experimental rickets. However, classical rickets does

not exist in this genus. Only under specific experimental conditions can bone lesions of rickets be produced in the rat (61). For production of experimental rickets in rats either calcium (34) or phosphorus (97, 170) or both (29) must be lacking in the diet. Usually D vitamins are excluded as well, although this is not absolutely necessary for the development of bone lesions (61). Experimental rickets in rats, therefore, differs significantly in this respect from the etiology of infantile rickets. The rat is reported to require only small amounts of D vitamins for normal bone growth (108). Also, the young rat has been reported to be able to absorb calcium in limited amounts in the absence of D vitamins (108).

To produce experimental rickets in rats, healthy rapidly growing individuals having a specific weight, a specific age and a genetic susceptibility for the development of rickets are required (61). The absolute amounts of calcium and phosphorus and the relative proportions of these minerals in the diet are the primary factors which determine the presence or absence and the severity of the bone lesions produced (94). Growth is essential to produce rickets (94). Given a diet which induces rickets the more rapidly the animals grow, the more severe are the lesions that are present (94). The diet must be, therefore, nutritious and complete in all respects for growth except for its content of calcium, phosphorus and D vitamins. For maximum growth, proteins of good quality must

be present in adequate amounts. Sufficient amounts of A vitamins and B vitamins as well as trace mineral must also be present (94).

The absorption of calcium and phosphorus from the intestine, so important in the production of experimental rickets in rats, has been found greatly influenced by a number of dietary factors (34, 55). The dietary factors which affect calcium absorption are the amount of calcium in the diet, the pH of the intestinal contents, the formation of insoluble calcium compounds by the phosphate and citrate ions, the presence of fatty acids and the lack of D vitamins. Dietary factors which influence phosphorus absorption are the amount of calcium in the diet, the pH of the intestinal contents and the formation of insoluble compounds with the calcium, beryllium, iron, lead, aluminum, strontium, magnesium, manganese and thallium ions (55, 72).

In the rat two distinct forms of experimental rickets can be produced (64, 114, 118). One form results when the ration fed is deficient in phosphorus and contains a normal or excessive amount of calcium. The second form is produced when the ration fed is deficient in calcium and contains a near normal amount of phosphorus. These rations produce bone lesions which correspond, in varying degrees, with the lesions of the ricketic infant. Rations deficient in phosphorus produce lesions which are reported to be identical to those of classi-

cal infantile rickets (95, 114, 115, 118). Rations deficient in calcium result in an atypical form of rickets which is believed by some authors to occur infrequently in the human infant (64, 118). The bones lesions of experimental calcium deficiency rickets have certain distinctive features. The arrangement of the trabeculae and blood vessels is the metaphysis is more orderly than in the phosphorus deficient form of the disease; bone destruction is increased greatly and mast cells are scattered in the intertrabecular spaces. Dietary imbalances other than the amounts of calcium and phosphorus may also produce bone lesions which resemble the phosphorus deficiency or the calcium deficiency form of experimental rickets. Severe phosphorus type of rickets can be produced by the presence of excessive amounts of either magnesium carbonate (114) or strontium carbonate (136) in the ration.

It should be noted that not all diets deficient in D vitamins and having unusual calcium-phosphorus balances regularly produce typical rickets. Also, in many instances, considerable individual variation is found using a single diet capable of producing rickets (118, 135).

Animals of the same age, on the same diet and under the same environmental conditions may have lesions which individually range from one extreme of severe classical rickets to the other extreme of severe osteoporosis or hypoplasia (118, 135).

Between these extremes, individuals may be present having intermediate forms with microscopic lesions of both diseases.

In the course of the study of experimental rickets in the rat, it was found the amount of D vitamins added to a specific ricketogenic diet could be quantitatively determined by the amount of mineralization in the healing bone. This healing could be demonstrated histologically, radiologically and biochemically. To develop a quantitative biological test for the presence of D vitamins, it was first necessary to be able to consistently produce a severe type of experimental rickets in which no mineralization of the epiphyseal cartilage plate occurred during the preparatory depletion period. McCollum et al. (93, 95) were able to develop such as biological tests which they described and named the "line test" because the new provisional zone of mineralization appeared as a line extending transversely across the bone with unossified cartilage on one side of it and unossified metaphysis on the other.

The success of the "line test" resulted from first, the formulation of a suitable ricketogenic diet and second, standardization of animals and methods of procedure. Their diet, McCollum Diet No. 3143, satisfied the first requirement, and since has been adopted by the U. S. Pharmacopeia as one of the official biological assay diets for D vitamins, Rachitogenic Diet No. 1 (29, 61). Other investigators also described suit-

able diets for the consistent production of experimental rickets. A similar diet to the McCollum Diet 3143 was described by Steenbock and Black (147) which contains fewer ingredients in slightly different proportions. This diet, Steenbock-Black Diet 2965, has been slightly modified and adopted by the U. S. Pharmacopeia as Rachitogenic Diet No. 2 (29, 61).

Sherman and Pappenheimer (133) described a third ricketogenic diet with which they could consistently produce experimental rickets. This diet, Sherman-Pappenheimer Diet 84, is biologically very poor; being deficient not only in the fat-soluble vitamins, but also B vitamins, vitamin C, protein and potassium. This diet is not officially recognized by the U. S. Pharmacopeia as a ricketogenic diet for biological tests (29).

The second requirement for the "line test" required that the animals be healthy, rapidly growing young rats between the ages of 21 and 30 days and weighing between 40 and 60 grams (95). During the preliminary period of growth these rats must receive no D vitamins in excess of those necessary for growth (61). The length of the depletion period for the production of bone lesions depends upon the age and weight of the rats. Steenbock et al. (148) demonstrated that an additional few days of age and 5 to 10 grams in the weight of the rats on a basal growth diet may increase the period required for the depletion of D vitamin stores and the production of rickets by several

weeks. The U. S. Pharmacopeia assay test for D vitamins specifically requires a depletion period of not less than 18 days and not more than 25 days (61). McCollum et al. reported depletion periods of 35 to 45 days (95) and 25 to 35 days (94) when using rats at ages of 35 to 40 days and 28 to 35 days respectively. At the end of this depletion period, rickets in these rats is clinically demonstrated by a distinctive, wobbly, unsteady gait and enlarged joints (123). When the rat moves off rapidly, it hops usually favoring one leg (123). At this time the rat should have histologic bone lesions of severe experimental classical rickets. These lesions correspond identically with those present in classical infantile rickets (122).

During the test period, accurate recording of the feed consumption is essential because a loss of appetite will result in starvation and spontaneous healing of the bone lesions (66, 95, 96). Also, if these rats remain on this same ricketogenic diet for a longer period of time than the necessary depletion period, rapid physical deterioration occurs which is accompanied by paralysis of the hind legs (131).

Macroscopic examination

Examination of these rats reveals emaciation and retarded growth (21). The hair coat is rough and uneven. The teeth are brittle (115). The cranial bones are soft. The costo-

chondral junctions of the ribs are enlarged, ovally shaped and displaced medially. Some animals have fractured ribs with callus formation (146). The spleen may be atrophic, enlarged or normal in appearance (135). Little or no body fat is present. The long bones are straight without fractures and enlarged at their ends. The femur and tibia are soft. Incision of the bone encounters little resistance until the knife has entered well into the shaft.

The cut surface of the femur and tibia reveals a broad zone of pale tissues, 2-4 mm. in width, lying between the epiphyseal cartilage and the shaft (135). The epiphyseal cartilage is increased in width and breadth. This enlargement is most marked at the periphery of the bone. The distal edge of the cartilage plate along the metaphysis is irregular in shape as a result of projecting tongues of cartilage.

Microscopic examination

Microscopic examination reveals little or no evidence of mineralization in the zone of preparatory mineralization (92, 93, 95, 96, 115, 135). Where mineralization occurs, it is fragmentary. This defect in mineralization is concurrent with the formation of osteoid tissue in the shaft. Compression of the cartilage cells and the trabeculae at the metaphysis may be observed as the result of pressure on the unossified tissues. The zones of vesicular and proliferating cartilage are in-

creased in breadth by a greater number of mature cartilage cells and in width by the accumulation of increased amounts of intercellular matrix between the cartilage columns. The normal vascular invasion of the epiphyseal cartilage is disturbed. Focal invasion of the zone of vesicular cartilage by capillaries from the marrow cavity is observed. Where these vessels have penetrated the interior of the cartilage mass, they branch in an arboreal pattern to form vascular "bushes", characteristic lesions of infantile rickets (115).

The metaphysis is composed chiefly of osteoid trabeculae, of blood vessels surrounded by small amounts of delicate connective tissue and a few marrow elements, of tongue-like protrusions of cartilage cells from the epiphysis and islands of cartilage cells (135). The trabeculae are broad. Many of them contain central cores of unossified cartilage and, in almost all of them, cartilage cells can be identified singly or in small groups in various stages of transition to a state indistinguishable from the cells of the osteoid itself. The osteoblasts covering the trabeculae are flattened, spread out like endothelial cells and have small nuclei. The osteoid of the trabeculae is for the most part laid down in the form of lamellae. The osteocytes vary in size, but the majority are small cells. The large prolongations of epiphyseal cartilage extending into the metaphysis retain the normal staining properties of cartilage.

Rickets and Osteodystrophy in Swine

Rickets, when the term is used in veterinary medicine and animal science, has four distinctly different definitions. Depending upon the writer, rickets may refer (1) to rickets in its classical concept (31), 2) to disturbances in bone growth which exhibit the bone lesions of classical rickets (87), (3) to disturbances in bone growth which result in defective ossification without regard to specific bone lesions (91, 148) and (4) to any skeletal disease which exhibits clinical symptoms or gross lesions of an osteodystrophy (157). The term rickets in the literature of veterinary and animal science has been loosely applied to express a variety of ideas. This lack of association of this term to a specific disease has resulted in many conflicting opinions and has made accurate correlation of the reported information difficult.

Rickets, if defined according to its classical concept in the human being, would refer to a specific disease of young swine caused by a deficiency of D vitamins when the diet contains adequate amounts of calcium and phosphorus. Affected pigs would have alterations which clinically, biochemically, radiologically and histopathologically correspond to those of the human disease. Classical rickets has never been described in swine.

Several authors, Joest and Zumpe (74) and Neiberle and Conrs (109) and others, have strongly questioned the existence of classical rickets in any domesticated animal. Runnells et al. (127) report never having demonstrated rickets in domestic farm mammals during examinations over a period of more than twenty years. These reports indicate that if classical rickets occurs in swine, it does so very rarely and then under very unusual circumstances.

Rickets may be defined broadly as any disturbance in bone growth which results in bone and cartilage lesions similar to those observed in human rickets. This definition of rickets may specifically apply to only deficiencies of D vitamins or also, broadly include calcium and phosphorus imbalances. Rickets, according to this definition, is a rare disease in swine. When it occurs, rickets in swine corresponds histologically to juvenile rickets in man (71). Porcine juvenile rickets has been reported to occur under natural farm conditions. However, few spontaneous cases of this disease have been adequately described.

Much research has been done on experimental juvenile rickets in swine, however, few of the reports on these experiments contain detailed descriptions, many lack essential information and most contain brief or no descriptions of the microscopic lesions produced. Microscopic examination is an essential re-

quirement for the study of any disease process.

Juvenile rickets has been reported in swine under a wide variety of experimental conditions. Initially, most of these experiments were designed to study only a single factor. However, analysis of the experiments frequently reveals multiple deficiencies and important factors unreported by the investigators, facts which markedly limit the value of these studies.

Experimental rickets in swine resembles, in many respects, experimental rickets in rats. However, the disease in swine has not been studied with sufficient detail to know what essential conditions are needed to consistently reproduce the disease in this genus. The same factors which influence the production of experimental rickets in the rat are believed to affect the production of experimental rickets in swine. Growth is essential (146). The experimental pigs must be young, healthy and growing rapidly (149). The exact age and weight requirements of these pigs are unknown. The majority of the experiments have been performed with weanlings from 6 to 9 weeks of age and weighing 35 to 60 pounds. These pigs have not been consistently susceptible to experimental juvenile rickets. Cunha (18) suggests most pigs at this age and weight have passed the critical period of growth in their life and carry sufficient body stores of vitamins and minerals to withstand many severe nutritional imbalances for long periods. Steenbock et al. (148, 149) have shown that the age and weight of rats

used in experiments are important in the production of experimental rickets and suggest this is the reason they failed to produce experimental rickets in their swine experiments. To produce experimental juvenile rickets, the diet must be adequate in all respects for good growth except the amount of calcium, phosphorus and D vitamins. This diet must also provide proteins of high quality, as well as sufficient amounts of A vitamins, B vitamins and trace minerals, to permit active bone growth.

Experimentally, juvenile rickets has been reported in swine fed rations deficient in calcium (31, 80), deficient in phosphorus (156), deficient in D vitamins (169) and having unfavorable calcium-phosphorus ratios (8). Experimental juvenile rickets in these animals has occurred with great variations in the percentage of affected animals, the severity and the length of the pre-patent period. Many attempts to produce experimental rickets in swine have failed completely. This variation in results has been reported both between breeds and between individuals of the same litters when the experimental and pre-experimental conditions were the same (75). Individual differences in the storage of D vitamins in the liver and the storage of calcium and phosphorus in the bones have been suggested to explain these variations (75, 161). However, little specific information has been presented to support these theories.

In experimental rickets in swine, reports have indicated

that a hereditary factor is also present (75). The full significance of the role of heredity is not known. It is possible that pigmentation of the skin, the rate of growth and body conformation are the principal inherited characteristics which have an important influence on the development of experimental rickets.

Experimental rickets, based on histopathological examinations, has been reported six times.

Zilva et al. (169) describe experimental juvenile rickets in eight out of ten pigs fed a ration deficient only in D vitamins. The pigs were sixty-two days old when placed on the experimental ration. The ration fed contained a calcium-phosphorus ratio of 1:06. The average daily consumption was 1.085 ounces of calcium and 1.065 ounces of phosphorus. These amounts are approximately five times more than those recommended by the National Research Council for swine. The diet consisted of toppings (wheat bran?) barley meal, dried separated milk, "animal charcoal", swedes (turnips) and chalk (calcium carbonate). The diagnosis of rickets was based upon histopathological examinations. This is the only detailed report of experimental rickets caused by D vitamin deficiency in swine when adequate amounts of dietary calcium and phosphorus were present.

Marek and Wellmann (87) studied extensively experimental juvenile rickets in swine. These experiments were based upon

variations in dietary calcium and phosphorus levels and the relationship of dietary acid-base balance to the production of rickets. Most of their experiments were also complicated by deficiencies in proteins, deficiencies in A vitamins and deficiencies in D vitamins. The lesions are described in detail in these experiments. However, the multiple deficiencies in these experiments prevent accurate interpretations of their results. The illustrations which accompany their text contain photomicrographs which demonstrate the presence of experimental juvenile rickets in some of their animals.

Theiler et al. (156) studied extensively the effect of calcium and phosphorus deficiencies in growing pigs. They reported experimental rickets could be produced in pigs fed a ration deficient in phosphorus with or without the presence of sunlight. They interpreted these results to mean the ration contained sufficient D vitamins for growth.

Rations deficient in calcium resulted in atrophy of the bone (osteoporosis), but not rickets. One pig on a ration deficient in calcium showed incipient osteodystrophia fibrosa.

When the ration was deficient in both calcium and phosphorus and the calcium-phosphorus ratio was normal, the pigs showed definite locomotor disturbances. The pig which received no sunlight was most severely affected. Microscopically, these pigs showed marked atrophy of the bone. A suggestion of rickets was found in two of four animals.

When the ration contained deficient levels of both calcium and phosphorus and the calcium-phosphorus ratio was unfavorable, no significant microscopic lesions were observed. When the acid-base balance of the ration was altered, Theiler was unable to produce lesions of experimental rickets using rations reported to be ricketogenic by Marek and Wellmann (1932).

The variance in results was believed to be the result of deficiencies in D vitamins which were present in the experiments of Marek (87), but not in the experiments of Theiler (156). The lesions produced in these experiments are briefly described and no photomicrographs accompany the text.

Elliot et al. (31) report experimental juvenile rickets in a small number of pigs using rations deficient in calcium. The disease could be prevented by the addition of calcium to the ration. They were unable to produce rickets with rations which were deficient in both calcium and the fat-soluble vitamins, A and D. The description of the microscopic lesions is too concise for adequate interpretation.

Loeffel et al. (85) report an extensive study of experimental rickets in swine. The diet fed in these experiments was deficient in both calcium and D vitamins. The descriptions of the lesions are brief and lack much essential information. No photomicrographs were published.

Kernkamp (80) reports microscopic lesions of experimental rickets in some of his animals fed rations deficient in calcium. The microscopic lesions are described very briefly. He reports that lesions of rickets, osteomalacia, and atrophy were present both singly and in various combinations in the animals examined.

McGowan (98) reports histologic evidence of a latent form of rickets in pigs from the tenth to the twentieth day of life. The clinical symptoms of the patent form did not usually appear until the animals were ten to twelve weeks of age. He regards rickets as the result of three primary factors; a deficiency of phosphorus in the diet relative to its protein content, the growth potential of the animal and its origination in the suckling pig. He considered the role of D vitamins as a secondary one.

Rickets, when used in non-medical discussions, frequently is applied to either any disturbance of bone growth which results in defective ossification witnout regard to specific bone lesions or to any disease of the skeleton which results in clinical symptoms or gross lesions of an osteodystrophy. Rickets, when used in this sense, has very little value since it gives indication of neitner the etiologic agent nor the causative factors which may have led to this disease. Without knowing the pathogenesis of the condition, therapeutic and prophylactic control is impossible.

Rickets, when the term is transposed from bone diseases of the human being to those of domesticated mammals, carries with it the implication, which is not true, that the same disease occurs as a result of the same etiology. Rickets in its classical concept is a specific disease of man and man alone. Because man is unique in his absolute requirements for D vitamins, his peculiar growth pattern, and his slow skeletal development, he is very susceptible to this disease.

Jones (76) proposed the term pseudorickets be used to distinguish the so-called "rickets" osteodystrophy of domesticated mammals from the true rickets in man. He suggests the osteodystrophy (pseudorickets) commonly observed is the result of multiple deficiencies of A and D vitamins and deficiencies or imbalances of calcium and phosphorus. He indicates pseudorickets is primarily caused by a deficiency of A vitamins.

Pseudorickets is a general term denoting a condition which has a close or deceptive resemblance to rickets (23). In man, it is used as a synonym for renal osteodystrophy (renal rickets) which histologically has similar bone lesions to those of classical rickets including excessive amounts of osteoid tissues, but has an etiology independent of D vitamins (23, 34).

Deficiencies of A vitamins in domesticated mammals has only a superficial resemblance to classical rickets and histologically is quite distinct (76). Renal osteodystrophy in

the dog is reported to be primarily a fibrous osteodystrophy (fibrous dysplasia) with a resemblance to osteitis fibrosa in man (68). Because bone sections are not taken routinely in cases of chronic nephritis, the presence of lesions resembling rickets may be undetected in many dogs.

The use of the term pseudorickets to mean osteopathy does not solve the semantic problem of rickets in animals. Until more basic research is conducted in bone diseases of animals, the term osteodystrophy, which has no implications in its wording to any specific syndrome or disease, is the prefered term (11).

Osteodystrophy is a metabolic disturbance in bone growth and results from many etiologic agents (11). Cases of bone disease which have no microscopic examinations should be reported as osteodystrophy. The term rickets should be applied only to those cases which have lesions which correspond to those described in man.

Osteodystrophy has been described frequently in swine both clinically and experimentally with and without microscopic examinations.

Kernkamp (80) reported osteodystrophy with severe disturbances in locomotion as a result of rations deficient in calcium. In this report, he indicates some animals had histopathologic lesions of rickets. Others exhibited atrophy and

some of the animals exhibited lesions of osteomalacia.

Steenbock et al. (148) in attempts to produce experimental rickets, produced osteodystrophy using rations deficient in D vitamins. Histopathologic examinations demonstrated imperfect calcification and an irregular metaphysis but failed to demonstrate any evidence of rickets. They took the attitude that rickets was a pathologic entity distinct from the abnormal relations observed in the mature animal only in that growth brings about the production of a special histologic picture, (classical rickets). They felt the practice of defining rickets solely on the basis of microscopic lesions distinct from its causative factors was archaic.

Sinclair (140) reported experimental production of "stiffness" in swine fed a diet deficient in calcium. No microscopic changes were described.

Sinclair (141) produced stiffness, clinically suggestive of rickets, with a ration deficient in calcium which could be markedly improved by the addition of sunlight, artificial irradiation and cod-liver oil. This condition could be prevented by the addition of 2 per cent of limestone to the ration.

Forbes et al. (39) reported individual variation in pigs receiving various deficiency rations. Gross bone deformities were observed on rations deficient in calcium fed in the pres-

ence of direct sunlight. No histopathologic examinations were made.

Hart et al. (58) produced severe locomotor disturbances, tremors and posterior paralysis in pigs fed a ration deficient in phosphorus. No microscopic examinations were reported.

Maynard et al. (89, 90) experimentally produced locomotor disturbances, "stiffness", in pigs fed a ration deficient in calcium which could be prevented by exposure of the animal to sunlight. Post mortem examination revealed osteodystrophy. Microscopic examinations were made and the microscopic changes are well described. There were no microscopic lesions of rickets in these animals.

Dunlop (26) reported that within normal areas no additional D vitamins were needed above the amount which may be present in cereal and protein meals of the basal ration.

He believed the true requirement of calcium and phosphorus must be dependent upon the rate of growth and the economy of gain. He found a calcium level of 0.45% of the ration and a phosphorus level of 0.6% were optimal in his experiment.

Stolte (151) found in his experiments no supplemental D vitamins were needed to prevent "rachitic lesions" in growing pigs fed a ration of corn and soybean oil meal, and confined in the absence of direct sunlight. The rations fed had a

range of 0.725% to 0.930% calcium and calcium-phosphorus ratios of 1.5:1, 2:1, and 2.5:1. No post mortem examinations and no microscopic examinations were made.

Bethke et al. (7) reported the growing pig has a definite fundamental requirement for D vitamins even in the presence of adequate calcium and phosphorus. They reported that with a calcium level of 0.6% and a phosphorus level of 0.45%, 90 U.S.P. units of Vitamin D were needed per pound of feed. No microscopic examinations were described. They observed definite variation in D vitamin requirements between groups of pigs on the same ration as well as individual pigs of the same breeding and the same pre-experimental nutritional level.

Pedersen (120) produced low serum calcium levels in young pigs fed a ration deficient in calcium. These pigs exhibited tremors and convulsions but no gross evidence of rickets. Arthropathy involving the articular surfaces of the joints was observed in some of these animals upon post mortem examination. Therapeutic treatment of these animals with injectable D vitamins resulted in recovery. No microscopic examinations were reported.

Shaw (131) studying osteodystrophy in swine noted that a deficiency of calcium in the ration resulted in loss of appetite, suppression of growth, onset of lameness and in some cases "rickets". He further noted that when a deficiency of calcium

did not produce clinical evidence of rickets, poor growth rates and poor feed utilization occurred.

Shaw (130) experimentally produced symptoms of "rickets" in pigs deprived of sunlight. The presence of sunlight resulted in rapid improvement. The calcium and phosphorus levels of the ration, the calcium-phosphorus ratio and the amount of mineral consumed were not reported. No post mortem or histopathologic examinations were described.

McGowan (98) reports histopathologic examinations made on pigs fed rations deficient in calcium revealed advanced osteoporosis with bone fractures. Cod-liver oil, sunlight and D vitamins would not prevent this disease. Bone lesions of rickets were entirely absent. When phosphate was added to the ration, the osteoporosis was less severe.

In this same report, McGowan states rickets occurs in pigs fed diets containing excessive amounts of calcium carbonate. In these cases calcium carbonate was added to balance the high phosphorus content of the concentrates used.

Zilva et al. (170) in experiments using suckling pigs fed from birth on a diet containing restricted amounts of fat-soluble vitamins (A and D) produced osteodystrophy. Histopathologic examinations did not reveal any lesions of rickets.

Golding et al. (46) produced a severe osteodystrophy in pigs fed rations deficient in A vitamins and fed rations deficient in A vitamins and calcium. The clinical appearances of these animals corresponded to field cases of "rickets". Histopathologic examination revealed osteoporosis. There was no excessive amounts of osteoid tissue to indicate the presence of rickets.

Kellermann et al. (78) also report experimental production of locomotor disturbances in pigs resulting from rations deficient in A vitamins and rations deficient in both A vitamins and calcium. The pigs in this experiment received abundant sunlight eliminating the possible presence of complicating deficiencies in D vitamins. No microscopic examinations are described.

Sandstedt (129) reports that deficiencies in A vitamins in swine produces symptoms of a diminished appetite, stiff and uncertain action of the hind legs, and tenderness of the muscles. In severe cases, paresis of the hind legs and inability to rise is observed. No microscopic examinations are reported.

Hart et al. (57) state growing pigs fed white corn and skimmed milk in dry lot, without any pasture, often develop "rickets". This is not due to a lack of calcium, but a lack of a fat-soluble vitamin. If the mixture is yellow corn and skimmed milk, there is much less danger of "rickets", because

yellow corn carries some of this needed vitamin. No microscopic examinations are reported. It is apparent in their experiments both D and A vitamin deficiencies were present.

Sheeny and Senior (132) report that a plentiful supply of D vitamins is required for assimilation of minerals by the pig. The absence of D vitamins even in the presence of abundant minerals results in "rickets", "cramps", "staggers" and convulsions. Deficiencies of minerals result in soft bones, unthriftiness, poor growth, depraved appetites and ultimately in "rickets". They studied extensively the effect of mineral deficiencies and report clinical evidence of rickets. However, no microscopic or post mortem examinations were done.

Orr and Husband (113) report that calcium and phosphorus are required in considerable quantities for normal bone development and therefore are both limiting factors for growth. Their experiments are mentioned only briefly and no microscopic examinations are recorded.

Johnson and Palmer (75) conclude that reduced calcium levels resulting from deficiencies in D vitamins have a pronounced effect on growth and feed consumption. They observe considerable variation between breeds and between pigs of the same breed in susceptibility to "rickets". They found a definite hereditary difference in the D vitamin requirements of swine associated with the color. White pigs are reported

less susceptible to D vitamin deficiency than colored pigs under similar conditions in the winter.

Jack (73) gives a case report of osteodystrophy in weaned pigs on a ration deficient in calcium, deficient in protein and having a very high phosphorus to calcium ratio. Severe locomotor disturbances were observed in these animals. The bones were soft and cut easily. There was no microscopic enlargement of the epiphyses or costochondral junction. The articular surfaces of the joints were normal in appearance. The description of the microscopic changes is very brief and lacks essential information. There was no definite lesions of rickets in these microscopic examinations.

Wahlstrom and Stolte (161) found there is no need for supplemental D vitamins in rations for growing pigs confined in the absence of sunlight and having calcium levels from 0.61% to 0.93%. In one experiment, they observed very poor growth and "rickets" in five out of eight pigs fed a corn-soybean oil meal ration with a mineral supplement fed free-choice. Additional D vitamins increased the rate of gain but did not give complete protection against rickets. Study of the amount of free-choice mineral and concentrates eaten revealed these pigs did not consume sufficient amounts of calcium or phosphorus to meet their minimum daily requirements for these elements. No microscopic or macroscopic examinations were reported.

Theiler et al. (1937) in their previously mentioned experiments produced osteodystrophy on rations deficient in phosphorus, rations deficient in calcium, and in rations deficient in both calcium and phosphorus. Histopathologic examinations revealed definite rickets in the phosphorus deficiency experiments and a suggestion of rickets in two out of four animals on rations deficient in both calcium and phosphorus. Rations deficient in calcium produced osteoporosis (bone atrophy).

Teague and Carpenter (152) report that a copper deficiency results in osteodystrophy in pigs. The affected pigs exhibit drawing under of the rear legs, crookedness of the forelegs, swelling in the region of the hocks, a turning in of the toes of the rear legs and extreme weakness of the carpal joints.

Follis (37) also reports a similar osteodystrophy resulting in pigs from copper deficiencies.

Clinical rickets in swine

The clinical appearance of juvenile rickets in swine is basically that of an osteodystrophy (70, 71, 87, 102, 126, 127, 142, 157). The early indications of this disease are retarded growth and hypoplasia of the skeleton. The animals are unthrifty and have rough haircoats. The ends of the bones enlarge so that the joints appear swollen. Nodular enlarge-

ment of the costochondral junctions occurs. The long bones may become distorted and deformed as the result of bending under pressure of the body weight. Multiple fractures of the long bones, the ribs and the vertebrae are frequently observed. The abdomen may be enlarged, distended and pendulous. The ribs become bent medially and a long shallow depression may form on either side of the chest. The pelvis is deformed with narrowing of its transverse diameter. The spine may be curved to either side, dorsally or ventrally.

The chief symptoms of juvenile rickets are disturbances in locomotion varying from a slight lameness or "stiffness" to inability to walk (10). The pigs assume unusual positions of the limbs when standing. The front legs may be directed slightly backward and flexed at the carpus or the pastern may be vertical so the pigs appear to stand on the tips of their toes. Later, the pigs may rest or move about on their knees. The hind legs are directed forward and the tarsal joint develops extreme flexion. Posterior paralysis as a result of fractured bones is a frequent symptom in severely affected animals. The pigs are usually recumbant and move with reluctance. In those cases where fractures of the femur or vertebrae occur, atrophy of the muscles is a sequelae. Death is frequently the result of bacterial bronchopneumonias. Excessive rooting or pica may be observed. Also, convulsions

and tetany may also occur.

Experimentally, juvenile rickets is reported to appear in swine one to three months after feeding of a deficient diet is begun. Rarely it has been reported at two weeks.

Macroscopic examination

Post mortem examination of pigs with juvenile rickets confirms the osteodystrophy observed clinically (70, 71, 87, 127, 142, 157). The epiphyses of the long bones and the costochondral junction are enlarged. Curvature, fracture or exostosis of individual bones may be observed. The osseous tissues are soft and are cut easily with a knife.

Longitudinal sections of the long bones reveal subperiosteal deposits of soft, vascular, spongy osteoid tissue. These deposits are thickest at the origin and insertion of muscles and tendons and at the sites of bending or fractures of the bone. The epiphyseal cartilage is from two to ten millimeters in breadth depending upon the age and severity of the disease. The cartilage is bluish in color, transparent and very vascular. The metaphysis is broad and irregular in shape. Cartilage prolongations, up to 3 millimeters in length, project towards the diaphysis. The zone of provisional mineralization which in healthy bone is visible as a distinct yellowish-white line, is absent or broken into irregular segments.

In mild cases mineralization is patchy, with more or less numerous ossified islets in the midst of unossified cartilage. The spongiosa of the metaphysis is redder than the normal appearance. In advanced cases, the spongiosa consists of reddish or yellowish-white, finely porous, fibrous connective tissue of uniform density, unmineralized and often studded with fine hemorrhages, containing cartilage processes. The bone marrow is of a red or brownish-red color. Loosening of the metaphysis may occur with separation of the epiphysis from the diaphysis. The spongiosa of the metaphysis may protrude laterally resulting in peripheral enlargement of the ends of the bone at this point.

These changes are most conspicuous at the sternal ends of the intermediate ribs, the proximal ends of the humerus and ulna, and slightly less severe in the proximal end of the femur, distal end of the humerus and the anterior and posterior ribs. The bones most frequently affected are, therefore, those in which a comparatively rapid growth is still proceeding at the time the disease develops.

Other conditions frequently present in swine affected with juvenile rickets are bronchitis, bronchopneumonia, catarrhal gastroenteritis and anemia. It must be remembered that the clinical appearance and gross lesions of juvenile rickets can not be differentiated from other osteodystrophic diseases of

swine without microscopic examinations. Manganese deficiency, (77, 103, 106, 121), copper deficiency, (36, 85, 162), hog cholera and hog cholera vaccination, (27, 28), swine influenza, (48, 49), vitamin A deficiency, (56, 129), and imbalances in calcium, (79, 80, 87), phosphorus (155, 156) and zinc (128) can also produce similar osteodystrophy.

Microscopic examination

Microscopic examination of the costochondral junctions and the epiphyseal ends of the long bones (70, 71, 127, 142) reveals enlargement of the epiphyseal cartilage plate, extensive vascular invasion of the cartilage plate, numerous active osteoblasts and an abundance of osteoid tissue. The zone of proliferating cartilage is increased in depth and width. The vesicular zone of the cartilage is distorted with twisting, bending, compressing and fracturing of the cell columns. The cell columns are irregular in length and tongue-shaped cartilage projections extend into the metaphysis. The invading blood vessels are surrounded by a sheath of fibrous connective tissue and are bordered by a layer of osteoid tissue in the cartilage plate. The provisional zone of mineralization is irregular and broken into segments. In very severe cases, this zone is completely absent.

The metaphysis consists of a broad zone of osteoid trabeculae which contain irregular central cores of bone and

cartilage cells. These trabeculae are bordered by a thick mantle of osteoid tissue and covered by a layer of active osteoblasts. These cartilaginous and osteoid trabeculae of the metaphysis may be fractured, twisted or compressed. The bony trabeculae of the diaphysis are decreased in number, widely separated and have borders of osteoid tissue in varying thicknesses. The periosteum is thickened, vascular and consists of excessive amounts of osteoid tissue. The bone marrow exhibits normal hematopoietic activity and contains variable amounts of delicate supporting fibrous connective tissue.

METHODS AND PROCEDURES

Twenty baby pigs were obtained from a local swine farm for this experiment. Their dams were commercial sows of a Yorkshire, Poland China, Duroc and Hampshire cross. The sire was a spotted Poland China boar. The pigs were selected at random from various litters. They weighed from two to five pounds at birth. Their color was white or white with black markings.

These pigs were taken from the sows at an age of 6 to 18 hours. This gave them ample opportunity to obtain colostrum before they were placed on the experimental diet. The pigs were moved by automobile from the centrally heated farrowing house where they were born to research rooms in the basement of the Veterinary Pathology building. To prevent chilling during this transfer, the pigs were carried in blanket-covered baskets.

During the experimental period, the pigs were housed in individual rabbit cages floored with masonite (Figure 1). The bedding which consisted of woodshavings, was changed daily. Fluorescent overhead lights, which did not radiate ultraviolet rays, were used for illumination. These lights were used only during periods of feeding and cleaning. The temperature was maintained at 90°F. by means of a thermostatically controlled drying oven (Figure 2). As the pigs became older, the tem-

perature was gradually lowered to that of the unheated room. Pans of water were kept in the oven to maintain a satisfactory humidity. The pigs were fed one ounce of a reconstituted synthetic milk diet four times a day using 100 cubic centimeter bottles with lamb nursing nipples. This diet consisted of a 15 per cent suspension of Ration A in warm tap water. When, after three days, it was found severe dehydration and deaths were occurring on this diet, the diet was modified by the substitution of 5 grams of dried skim milk for 5 grams of Ration A. The amount fed was increased to one and a half ounces per feeding and the feeding times to five times a day. These modifications were made to dilute the amount of electrolytes present and increase the fluid consumption. As the pigs became older and larger, the amount fed and the time intervals were increased. When scouring occurred, 1 gram of infant's rice cereal[1] was added to the reconstituted synthetic milk for added bulk. This modified diet was fed individually and quite effectively controlled the diarrheas in these pigs. The pigs which reached four weeks of age were fed ration B free choice in addition to the liquid synthetic milk diet.

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[1]Gerber Products Company, Fremont, Michigan

The diets fed consisted of the following ingredients:

	Ration A	Ration B
Dried skim milk	40.00 lbs.	20.00 lbs.
Cane sugar	16.00	15.55
Lactose	5.00	-
Ground yellow corn	10.00	41.00
Solv. soybean oil meal (44%)	-	15.00
Alpha (soy) protein[1]	7.50	-
Dried brewer's yeast (U.S.P.)	2.00	2.00
Stabilized lard	4.00	-
Dried beet pulp	2.00	2.00
Dried sweet whey	10.50	-
Iodized salt	0.50	0.50
Dicalcium phosphate	0.75	1.80
Special vitamin diet	-	-
Fortification mixture	2.20	-
Aureomycin (T. F.-15)	0.25	-
Trace mineral mix	0.10	0.10
Calculated calcium content	0.621	0.741
Calculated phosphorus content	0.684	0.786

Special vitamin diet fortification mixture without D vitamins:

	Grams/100 lb. of mixed diet
Vitamin A concentrate (200,000 units per gram)	4.5
Alpha tocopherol	5
Ascorbic acid	45
Inositol	5
Choline chloride	75
Menadione	2.25
p-Aminobenzoic acid	5
Niacin	4.5
Riboflavin	1.0
Pyridoxine hydrochloride	1.0
Thiamine hydrochloride	1.0
Calcium pantothenate	3

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[1]Nutritional Biochemicals Corporation, 21010 Miles Ave., Cleveland 28, Ohio

	mgms/100 lbs. of mixed diet
Biotin	20
Folic acid	90
Vitamin B_{12}	1.35
Trace mineral mix (guaranteed analysis)	**ppm or micrograms per pound**
Iron	80,000
Copper	6,380
Manganese	40,630
Cobalt	2,850
Zinc	32,120
Iodine	1,370

The trace mineral mixture was added to both Ration A and Ration B at the rate of 2 pounds per ton of feed. The sodium chloride content of Ration A was 0.7 per cent. The A vitamin content of Ration A at the conclusion of this experiment was assayed at less than 1000 units per pound of feed. The ratio of calcium to phosphorus in both Ration A and Ration B was approximately 1:1.

The clinical appearance of the pigs was noted at the time of feeding. Any abnormality was recorded. Animals which died were examined as soon as possible after death. Gross lesions were recorded and tissues were preserved by fixation in 10 per cent formolized saline and stored in 70 per cent ethyl alcohol for later histopathologic examination.

Pigs which survived the initial three weeks were euthanized at 21 day intervals to determine the effect of the experi-

mental diet on their bone development. The experiment was terminated at 63 days. At this time, it was apparent that normal growth was resumed and no additional osteodystrophy was occurring.

Control Experiments

To provide environmental controls, eleven 30 gram rats, age 22 days, and twenty-four one-day-old chicks were fed a standard ricketogenic diet, U.S.P. Rachitogenic Diet no. 2, and raised in the same rooms as the pigs. An additional eleven 30 gram rats and twenty-four one-day-old chicks were fed the experimental pig diet, ration A, to determine its effect on their growth and bone development. Five 30 gram rats were fed the standard U.S.P. vitamin A deficient test diet. Three 30 gram rats were fed a complete commercial rat diet. These animals were housed, fed and watered using standard laboratory procedures. Their clinical appearance was noted and recorded at the time of feeding. Representative animals from each group were euthanized and examined at definite intervals of time to demonstrate their bone development. Tissues were fixed and stored in the same manner as specimens from the experimental pigs. These control experiments were terminated at ten weeks.

Histopathologic techniques

Tissues saved for histopathologic examination were the

costochondral junction of the fifth, sixth and seventh ribs, the distal end of the femur and the proximal end of the tibia. At the time of necropsy, the bones were sectioned[1] longitudinally to permit penetration of the fixative. After preliminary fixation in neutral 10 per cent formolized saline for 48 hours in a 40°F. refrigerator, the tissues were washed and trimmed[1] in sections 3 to 6 mm. thick and approximately 20 mm. square. These tissues were placed in fresh formolized saline and returned to the refrigerator. After an additional 72 to 96 hours fixation, these tissues were washed and stored in 70 per cent ethyl alcohol until the end of the experiment.

These tissues were decalcified using a 30 per cent formic acid-ion exchange resin solution (24, 48). Decalcification of the 3 mm. sections was complete in 18 hours. After 18 hours of fixation thicker sections were trimmed[2] to a thickness of 2 to 3 mm. and returned to the decalcifying solution for an additional 24 hours. This technique resulted in rapid decalcification as well as the preservation of excellent cytologic detail.

When the tissues were adequately decalcified, they were washed for 24 hours in running tap water to remove the excess formic acid. After washing, they were trimmed[2] with a sharp

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[1]Initial trimming of the bones was done with a jeweler's saw having a number "0" saw blade with 64 teeth per inch

[2]Final trimming of bones was done with a sharp single edge razor blade

razor blade to give a smooth surface for sectioning. They were then dehydrated, cleared and infiltrated with celloidin and paraffin using the double infiltration method described in the "Manual of Histologic and Special Staining Technics" of the Armed Forces Institute of Pathology. The tissues were embedded in Altman's paraffin.

The tissues were infiltrated and embedded as follows:

1.	Alcohol, 70%	24 hours
2.	Alcohol, 70%	24 hours
3.	Alcohol, 95%	24 hours
4.	Alcohol, 95%	24 hours
5.	Alcohol, absolute	24 hours
6.	Alcohol, absolute	24 hours
7.	Ether-alcohol[a]	24 hours
8.	Celloidin, 2%	3 days
9.	Clearing oil mixture[b]	2 days
10.	Benzene	24 hours
11.	Benzene	24 hours
12.	Paraffin[c]	1 hour
13.	Paraffin[c]	1 hour
14.	Paraffin[c]	1 hour
15.	Paraffin[c]	1 hour
16.	Embed. in paraffin[c] and cool quickly	

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[a]Ether-alcohol was a solution composed of equal parts ether and absolute alcohol

[b]Clearing oil mixture was made up as follows:

Chloroform	4 parts
Oil of origanum	2 parts
Oil of cedarwood	4 parts
Absolute alcohol	1 part
Phenol	1 part

[c]Altman's paraffin mixture consists of the following ingredients:

Paraffin	850 grams
Stearic acid	100 grams
Beeswax	50 grams

The embedded tissues were sectioned as seven microns thickness and fixed to glass microscope slides using standard histological techniques.

All the tissues were stained by two methods; a routine hematoxylin and eosin stain and a modified Bock-Hansen calcium stain. The latter stain was used to distinguish between osteoid tissue and calcified bone.

The routine hematoxylin and eosin staining procedure was accomplished as follows:

1.	Xylene	3 minutes
2.	Xylene	3 minutes
3.	Alcohol, absolute	3 minutes
4.	Alcohol, 95%	3 minutes
5.	Alcohol, 70%	3 minutes
6.	Water, distilled	3 minutes
7.	Mayer's hematoxylin	10 minutes
8.	Water, running tap	10 minutes
9.	Ethyl eosin, 0.25%	5 seconds
10.	Alcohol, 95%	3 minutes
11.	Alcohol, absolute	3 minutes
12.	Alcohol, absolute	3 minutes
13.	Alcohol, absolute	3 minutes
14.	Xylene	3 minutes
15.	Xylene	3 minutes
16.	Xylene	3 minutes
17.	Mount in synthetic resin mounting medium and coverslip.	

A modified Bock-Hansen calcium stain was done according to the following procedure:

1.	Xylene	3 minutes
2.	Xylene	3 minutes
3.	Alcohol, absolute	3 minutes
4.	Alcohol, 95%	3 minutes
5.	Alcohol, 70%	3 minutes
6.	Water, distilled	3 minutes

7. Harris hematoxylin 30 minutes
8. Water, running tap 10 minutes
9. Glacial acetic acid--glycerin (solution of equal parts of each) was used to differentiate each slide until the desired intensity of stain was obtained.

10. Water, running tap 10 minutes
11. Water, tap saturated with lithium carbonate 10 minutes
12. Ethyl eosin 5 seconds
13. Alcohol, 95% 3 minutes
14. Alcohol, absolute 3 minutes
15. Alcohol, absolute 3 minutes
16. Alcohol, absolute 3 minutes
17. Xylene 3 minutes
18. Xylene 3 minutes
19. Xylene 3 minutes
20. Mount in synthetic resin mounting medium and coverslip.

RESULTS

Clinical Examinations

Clinical examinations of the pigs during the course of this experiment resulted in the following observations:

Pig 1(30), 3rd day: A severe diarrhea, rapid dehydration and death occurred within 12 hours after this pig was first noticed sick.

Pig 2(30), 3rd day: Severe dehydration without a diarrhea occurred in this pig. Death occurred without any other symptoms.

Pig 3(30), 4th day: Convulsions were exhibited ten minutes after feeding. Paddling of the front and hind legs and excessive salivation were observed. Death occurred three hours after symptoms first appeared.

Pig 4(30), 4th day: Edema of the subcutaneous tissues was noted in region of the thighs. This pig showed convulsions after being fed and died a few minutes later.

Pig 5(30), 11th day: Depression, incoordination and a slight dyspnea were observed. Four hours later, this pig was unable to eat or stand. It died 10 hours after first showing symptoms of illness.

Pig 6(30), 11th day: Dehydration and a rough hair coat were present. The appetite was good. He had an alert attitude. 16th day: His respirations were feeble. Severe de-

pression and weakness were exhibited. This pig was unable to eat or stand and died on the 16th day.

Pig 1(3), 21st day: Slight edema of the ventral underline was observed. The hair coat was rough and the ears were thick and rolled, Figure 3. 22nd day: Severe edema of the ventral underline and the stifles was present, Figure 7. This animal became excited and incoordinated immediately after eating. Recovery from the incoordination was rapid. 29th day: The subcutaneous edema had gradually been absorbed and now these tissues appeared normal. A dry, yellow serum exudate remained on the surface of the skin in the affected areas, Figure 8. 46th day: The weight of this pig was 6 pounds. Its appetite was good. Severe suppression of skeletal growth was apparent. 56th day: The appetite of this animal was markedly increased and his present rate of growth was rapid. He was aggressive in his attitude and his appearance was thrifty (Figure 13). 63rd day: This animal weighed 17 pounds. A slight osteodystrophy was observed. This pig stood with the elbows rotated laterally from the thorax, distinct flexion of the carpal joints and marked extension of the fetlock joints. This caused him to stand on the tips of his toes. The metacarpal bones turned medially. The stifle and hock joints of this pig were markedly extended causing him to be straight hocked and to stand on the tips of the toes of his hind feet. This experiment was terminated on this 63rd day with euthanasia by electrocution.

Pig 2(3), 18th day: This pig was unthrifty in appearance. The hair coat was rough. The ears were thick and rolled at their tips (Figure 4). Slight edema of the hind legs and ventral underline was observed. An osteodystrophy with marked turning of the hocks medially was present. 46th day: This pig weighed 11 pounds. Marked distortion of the front and hind legs was observed. The hocks were distinctly turned medially. The elbows were rotated in a lateral direction and the metacarpal region curved distinctly towards the midline. The appetite was good. Growth had been rapid since the 28th day. His attitude was alert and aggressive, (Figure 14). 63rd day: The weight was 27 pounds. Severe osteodystrophy was present. The elbows were distinctly bowed laterally from the thorax and the carpal joint was slightly overextended. This caused the animal to stand on the soles of his front feet. The stifle joint was lower than normal, had rotated laterally and was slightly extended. The hock joint was slightly flexed and the metatarsal region had rotated laterally at an acute angle from the midline. The digits of the hind feet turned sharply towards the midline so that the weight of the animal rested on the soles of the lateral chief claw (Figures 16, 17, 18). This experiment was terminated by euthanasia on this date.

Pig 3(3), 14th day: A mild osteodystrophy was noted. The digits of the front feet turned medially. 16th day: Severe edema of the thighs and ventral underline was present. This

pig had a harsh infrequent cough. The appetite was good. Penicillin, 25,000 units, and dihydrostreptomycin, 50 mg. were given without any noticeable response. Death occurred on the 16th day.

Pig 4(3), 8th day: Severe dehydration and a profuse watery diarrhea were observed. The appetite was good but this animal was very weak. 10th day: This pig was unable to eat and died.

Pig 1(7), 16th day: Edema of the thighs was observed. 19th day: Severe edema of the thighs, stifles and ventral abdominal wall was observed. The appetite was good. The hair coat was rough. 31st day: This animal was unable to eat or stand and showed convulsions. The ears were thick and rolled at the tip. Severe edema of the thighs and ventral underline was present. That evening the pig appeared much better but was found dead during the morning feeding period.

Pig 2(7), 4th day: Weakness of the hind legs was noticed. Dehydration without a diarrhea occurred in this animal. 6th day: The condition of the pig appeared improved; however death occurred without additional symptoms on the 6th day.

Pig 3(7), 14th day: Weakness of the hind legs was apparent. 15th day: Marked edema of the stifles and hocks occurred. The appetite was good. 18th day: Pronounced edema of the stifles with folds in the skin of the hocks was evident (Figures 6 and 9). The hocks rotated distinctly towards the midline.

Mild incoordination was present. This pig coughed occasionally. 24th day: The edema was gone (Figure 10). The osteodystrophy of the hocks remained. 42nd day: This experiment was terminated with euthanasia by electrocution. The only abnormality observed was the moderate distortion of the hocks.

Pig 4(7), 13th day: This pig was found dead.

Pig 1(9), 15th day: The hair coat was rough. Slight edema of the thighs was observed. An infrequent cough was noted. The appetite was good. 18th day: Severe edema of the head was observed, especially in the throat and occipital regions (Figure 5). A severe moist cough was present. The appetite remained good. 19th day: Severe dyspnea was observed. The umbilicus was edematous and extensive amounts of a dry serum exudate was present on the surface of the skin of the thighs (Figure 11). Penicillin, 12,500 units, and dihydrostreptomycin, 25 milligrams were administered subcutaneously. Death occurred six hours after the antibiotics were administered.

Pig 2(9), 13th day: This pig was unable to stand or eat. Death resulted 12 hours after symptoms were first observed.

Pig 3(9), 6th day: The pig was alert and normal in appearance. 500,000 units of calciferol, vitamin D_2, were given subcutaneously. 13th day: Incoordination of the hindlegs was noticed. Slight edema of the thighs appeared. 14th day: Depression and inability to eat or stand were observed. Death

occurred within six hours after the pig became too weak to eat or stand.

Pig 4(9), 6th day: This pig was alert and healthy in appearance. 500,000 units of calciferol, vitamin D_2, were given subcutaneously. 13th day: Slight incoordination of the hind-leg was apparent. The appetite was good. No edema was present. 15th day: This pig showed depression, weakness and inability to stand or eat. Death occurred six hours after the animal became too weak to eat.

Pig 5(9), 12th day: Incoordination and weakness were apparent. This pig became rapidly depressed and soon was unable to eat. Death occurred within 12 hours after the animal became unable to eat.

Pig 6(9), 12th day: Slight incoordination of the hind legs was noticed. The appetite was good. 13th day: This pig was unable to stand, became progressively weaker and died during a period of 6 hours.

Clinical observation of the control animals during the course of this experiment revealed the following information.

The synthetic milk diet, Ration A, when fed to chicks and rats in the concentrated form was very diuretic. The chicks had a profuse diarrhea. The rats had no diarrhea. Large amounts of water were consumed by both groups of animals. The damp bedding in their cages required daily changing to prevent

extensive mold growth. The addition of one part of infant's rice cereal[1] to four parts of Ration A was made to increase the bulk in the diet of the chicks. This procedure markedly reduced the diarrhea in this group. Since no diarrhea occurred in the rats, the diet was fed to them in the concentrated form. In spite of the diuretic properties of Ration A, both the rats and chicks grew rapidly and showed no other clinical abnormality. The chicks feathered rapidly and were alert and aggressive. The rats grew rapidly, had smooth haircoats and were active and alert.

The rats and chicks on the ricketogenic control diet, U.S.P. Rachitogenic Diet no. 2, grew poorly. Early in the experiment, their appetite was good. Later, their appetite was variable with alternating periods of increased and decreased feed consumption.

Day-old chicks on this control diet became weak, unable to stand and began dying after the twelfth day of life. The addition of one part of Ration A to four parts of the ricketogenic control diet caused a pronounced improvement in the appearance of these weak chicks. Recovery was rapid. Within twenty-four hours, most of these chicks appeared normal. Four chicks exhibited weakness in their legs, inability to stand or lameness and were euthanized with chloroform. The bene-

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[1]Gerber Products Company, Fremont, Mighigan

ficial effect of Ration A was believed because of its being a readily utilizable source of vitamins, other than the D vitamins, trace minerals, proteins, carbohydrates and fats.

Three days after being placed on the diet containing Ration A, these chicks were again fed the original ricketogenic control diet. No deaths occurred until the thirty-fourth day of life when four chicks died between the thirty-fourth day and the thirty-seventh day. All the chicks showed depression, weakness, drooping of the wings and ruffled feathers. Again rapid recovery occurred when the chicks were placed on a mixture of one part of Ration A to four parts of the ricketogenic control diet. After four days of this modified ration, the chicks appeared to be normal and were returned to the original ricketogenic diet. No more deaths occurred during the remainder of the experiment which was terminated on the fifty-seventh day.

Throughout the experiment, the chicks on the ricketogenic control diet had a very slow rate of growth, were slow in feather development and lethargic in attitude.

The rats on the ricketogenic control diet had rough haircoats, were very small for their age and were reluctant to move about in their cages. No spontaneous deaths occurred in these animals during this experiment. These rats were euthanized with chloroform at definite time intervals and examined.

The rats consuming the commercial ration for laboratory rats were normal in appearance. They grew rapidly and were aggressive, active and alert. Their haircoats were smooth and glossy.

The rats on the U.S.P. Vitamin A deficient diet were normal in appearance during the first ten days of the feeding period. They grew rapidly, had smooth haircoats and were active. On the fifteenth day, an exudative conjunctivitis was noticed which gave them characteristic rings around the eyes. The rate of growth began to decrease and the haircoats became rough. Three rats on this diet were euthanized with chloroform on the seventeenth day. The remaining two rats died on the thirty-second and thirty-third days of the experiment.

Macroscopic Examinations

Macroscopic examination of the pigs which died or were euthanized during the course of this experiment resulted in the following observations.

Pig 1(30): Dehydration, congestion of the lungs and a crystalline precipitate in the renal pelves were observed. The stomach was filled with ingesta.

Pig 2(30): Macroscopic examination revealed the same findings as pig 1(30) described above.

Pig 3(30): Extensive edema of the subcutaneous and skeletal connective tissues was noted. The lungs were congested. The stomach was filled with ingesta.

Pig 4(30): Extensive subcutaneous edema was present which also involved the skeletal connective tissues. The lungs were congested. A catarrhal gastroenteritis was present.

Pig 5(30): A fibrinous pleuritis, peritonitis and pericarditis were present. The ventral edges of the apical and cardiac lobes of the lungs were atelectatic. Severe edema of the subcutaneous and skeletal connective tissues were observed. This edema was most prominent along the ventral abdominal wall, the neck, the back, the legs and the thighs.

Pig 6(30): A fibrinous pleuritis was present. The ventral edges of the apical and cardiac lobes of the lungs were atelectatic. Severe edema of the subcutaneous and skeletal connective tissues was observed. This edema was especially prominent in the ventral abdominal tissues, the back, the thighs and the neck.

Pig 1(3): Slight distortion of the metacarpals was apparent. The ribs were soft and pliable. The long bones were dense and difficult to incise with a post mortem knife. There was no gross distortion of the epiphyseal line. The skeletal and cardiac muscle was pale brown.

Pig 2(3): Marked distortion of the hocks, metacarpals

and metatarsals was noted. No gross abnormalities were observed in the epiphyseal lines. This animal appeared normal in all other respects.

Pig 3(3): The ventral borders of the apical and cardiac lobes of the lungs were atelectatic. Severe edema of the subcutaneous and skeletal connective tissues was observed. This edema was most severe in the thighs and ventral abdominal wall. The heart was flaccid. The bones were easily incised. Grossly, the epiphyseal lines appeared more prominent and broader than normal.

Pig 4(3): Broad, prominent white lines were observed at the costochondral junctions and the epiphyseal lines. The ureters and the renal pelves were dilated and contained a thick gelatinous fluid which clotted when exposure to the air was allowed. A catarrhal gastroenteritis was present.

Pig 1(7): Edema of the subcutaneous connective tissues of the thighs and ventral abdominal wall was present. No other lesions were observed.

Pig 2(7): Congestion of the lungs and kidneys was noted. The renal pelves were dilated and contained a thick gelatinous fluid. Broad prominent white lines were observed at the costochondral junctions and at the epiphyseal lines of the long bones (Figure 24).

Pig 3(7): A chronic bronchopneumonia primarily involving the apical and cardiac lobes was present. No bone lesions

were observed.

Pig 4(7): Extensive severe edema of the subcutaneous and skeletal connective tissues was present. This edema was most pronounced in the thighs, at the back of the head and in the ventral abdominal wall. Excessive pleural fluid was present. The bones were hard and difficult to incise with a knife.

Pig 1(9): A severe bilateral broncho-pneumonia was observed. Excessive pleural and pericardial fluids were present. Severe edema of the thighs, ventral abdominal wall and cervical region was noted.

Pig 2(9): Extensive severe edema of the subcutaneous and skeletal connective tissues was present. This edema was most prominent in the shoulders, thighs and legs of this animal. The ventral margins of the apical and cardiac lobes of the lungs were atelectatic.

Pig 3(9): Extensive severe edema of the subcutaneous and skeletal connective tissues was noted (Figures 19 and 20). This edema was most severe in the ventral abdominal wall, (Figure 20), the legs and the cervical regions (Figures 21, 22 and 23). The ventral edges of the cardiac and apical lobes of the lungs were atelectatic.

Pig 4(9): Extensive edema of the subcutaneous and skeletal connective tissues was observed. This edema was most extensive in the pectoral region, the head and the neck. The ventral edges of the cardiac lobes of the lungs were atelectatic.

Pig 5(9): Extensive subcutaneous and skeletal connective tissue edema was present. This edema was most severe in the ventral abdominal wall especially in the region of the prepuce, the thighs, the back and the occipital region of the head.

Pig 6(9): Extensive edema of the subcutaneous and skeletal connective tissues was present. This edema was most severe in the legs, thighs, neck, head and back.

Post mortem examination of the control chicks and rats which died or were euthanized with chloroform during the course of this experiment resulted in the following observations.

Chicks fed experimental pig "synthetic" milk, Ration A, had no gross lesions. The bones of these birds were broken easily and were incised easily with a knife. No osteodystrophy was apparent.

Rats fed the experimental pig diet, Ration A, had no gross lesions. These animals were indistinguishable in appearance from the rats fed a complete commercial laboratory feed.

Chicks fed the control ricketogenic ration, U.S.P. Rachitogenic Diet no. 2, which were euthanized or died on the twelfth and thirteenth day of life, had soft bones which were easily bent and cut. The birds were emaciated and had pale yellow livers. Chicks which were examined on the eighteenth day, twentieth day, twenty-eighth day and fifty-eighth day of life

were small for their age, were emaciated and had soft bones which bent and cut easily. No osteodystrophy was apparent.

Rats fed the control ricketogenic diet, U.S.P. Rachitogenic Diet no. 2, were small for their ages, were emaciated and showed a definite osteodystrophy. A thick broad zone of pale red tissue could be seen between the epiphyseal cartilage and the diaphysis of the femur and tibia.

Rats fed the U.S.P. Vitamin A deficient diet which were euthanized on the thirty-first day of life, at this time had an exudative epidermitis in the form of a ring around the eyes. No other lesions were observed. The rat which died on the forty-sixth day had a hemorrhagic cystitis. On the forty-seventh day, the final rat on this diet died with a pyelonephritis. No osteodystrophy was observed in these rats.

A comparison of the growth rate of the chicks and rats fed Ration A, U.S.P. Rachitogenic Diet no. 2 and the commercial laboratory rat diet is summarized in the following table.

Animals	Age	Ricketogenic control diet	Ration A	Commercial lab. rat diet
Rats	55 days	56.9 gm.	204 gm.	181.8 gm.
	93 days	71.3 gm.	225 gm.	237.8 gm.
Chicks	20 days	58.0 gm.	120.0 gm.	
	28 days	64.5 gm.	193.2 gm.	
	58 days	127.4 gm.	522.0 gm.	

Microscopic Examinations

Microscopic examination of the tissues at the costochondral junction[1] of the sixth rib of the pigs used in this experiment resulted in the following observations.

Pig 3(30)[2], age, 4 days: A definite osteodystrophy was present at the costochondral junction of this animal. The resting and proliferating cartilage zones were normal in appearance. The vesicular zone was very broad, averaging 56 cells and had irregularly shaped cartilage projections, up to 157 cells in breadth, which extended towards the diaphysis (Figure 26). Distortion of the cartilage cell columns of the vesicular zone resulted from increased amounts of intercellular cartilage matrix and irregularities in the size and shape of the individual chondrocytes. The zone of vascular invasion and cartilage destruction was irregular as the result of the projecting tongues of cartilage (Figure 39). The blood vessels were actively congested and penetrated the metaphysis in an irregular manner. Ossification of the cartilage was normal.

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[1]Lesions observed at the distal end of the femur and the proximal end of the tibia are not reported because microscopic examinations revealed almost identical changes to those observed in the ribs. The only differences observed were intensity of the bone changes and individual variances of the bone.

[2]The death of pigs 1(30) and 2(30) on the third day was believed to be too early for bone disturbances to result from the diet fed. Therefore, no bones were saved for histopathologic study.

The cells of the vesicular cartilage in the areas of poor vascularity were undergoing hydropic degeneration and the cartilage matrix had become homogeneous and stained deeply with eosin. Focal areas of cartilage were necrotic. These degenerative cartilage changes were accompanied by proliferation of the osteoblasts in an irregular cell group, numerous multinucleate syncytial cells (Figure 43), numerous mononuclear macrophages, proliferation of a delicate reticular connective tissue and edema in the intertrabecular spaces. Serous inflammation was present in these areas (Figure 42).

The primary spongiosa consisted of irregularly arranged osteochondral trabeculae with thin borders of osteoid (Figures 52, 53 and 54). Numerous syncytial cells containing pyknotic nuclei were scattered throughout the intertrabecular spaces among the edematous reticular connective tissue fibrocytes. The secondary spongiosa was composed of thin trabeculae. Thick irregularly shaped trabeculae,bordered with osteoid layers of variable thicknesses formed the cortex of the diaphysis. The vessels of both the secondary and primary spongiosa were actively congested. Bone marrow growth was suppressed. The periosteum was thin and a slightly increased number of fibroblasts and osteoblasts were present. Numerous multinucleate syncytial cells were found in the periosteal connective tissues.

Pig 4(30), Age, 4 days: The zones of resting and pro-

liferating cartilage were normal in appearance. The vesicular cartilage zone was narrow and averaged 17 cells in breadth. Regular vascular invasion and cartilage destruction was occurring (Figures 28 and 31). Mineralization was occurring in a normal pattern. The blood vessels contained very few erythrocytes. Irregularly shaped thin spicules of ossified cartilage matrix formed the primary spongiosa. Osteoblasts were few in number. Little or no bone deposition was occurring. Delicate reticular connective tissue filled the intertrabecular spaces. Osteoid tissue was present as a thin layer or absent from the surface of osteochondral trabeculae. Multinucleate syncytial cells were few in number. Scattered irregularly shaped thick osteochondral trabeculae formed the secondary spongiosa. The cortex was thin and was composed of a few irregularly shaped thick trabeculae. Bone marrow growth was suppressed. The periosteum was thin and showed no increase in osteoblasts or fibroblasts.

Pig 5(30), Age, 11 days: The resting and proliferating cartilage, zones were normal in appearance. The vesicular cartilage zone was narrow and averaged 13 cells in breadth (Figure 33). Distortion of the cartilage columns in the vesicular zone resulted primarily from irregularities in the size and shape of the chondrocytes. The pattern of vascular invasion and cartilage destruction was regular. Mineralization was occurring in a normal pattern. Osteoblasts were numerous

and the active cuboidal form. Numerous syncytial cells were present along the zone of cartilage breakdown. A few foci of necrotic of cartilage cells were present. These areas appeared to be resistant to vascular invasion and projected a short distance into the primary spongiosa. Blood vessels were actively congested. The primary spongiosa consisted largely of a lattice of osteochondral trabeculae. These trabeculae were composed of a large central core of calcified cartilage matrix covered with a thin layer of bone or osteoid. The secondary spongiosa was composed of scattered irregularly shaped and sized osteochondral trabeculae. The intertrabecular reticular connective tissue was edematous. The blood vessels were actively congested. Suppression of bone marrow growth was apparent. The cortex was formed by thick, irregularly shaped and sized trabeculae (Figure 55). The periosteum was thin and showed no increase in osteoblasts or fibroblasts (Figure 61).

Pig 6(30), Age, 16 days: The resting and proliferating cartilage zones were normal in appearance. The vesicular cartilage zone was narrow and averaged 12 cells in breadth (Figure 25). Increased amounts of intercellular cartilage matrix was observed in the vesicular zone. Almost complete disruption of the normal columnar arrangement of the vesicular cartilage zone occurred as a result of the great irregularity in the size, shape and distribution of the chondrocytes.

Vascular invasion and cartilage destruction were irregular in arrangement and formed a narrow zone. The zone of vesicular cartilage contained focal areas of cartilage necrosis (Figure 39). Blood vessels were few in number, were scattered in the intertrabecular spaces and contained few erythrocytes. Osteoblasts were few in number and were principally of the inactive squamous or fibroblastic type (Figure 46). A few irregularly sized and shaped osteochondral trabeculae formed the primary and secondary spongiosa. Osteoid tissue was present as thin layers bordering the trabeculae or was absent. A few multinucleate syncytial cells were present. Some trabeculae in both the primary and secondary spongiosa contained foci of necrotic and ossified cartilage cells. The growth rate of the bone marrow was suppressed. Blood vessels were passively congested. The cortex was thin. The periosteum was thin and contained few osteoblasts and fibroblasts (Figure 62).

Pig 1(3), age, 63 days: The resting cartilage zone was normal in appearance. Active cartilage cell multiplication and growth were occurring in the zone of proliferating cartilage. Twisting of the cartilage columns resulted in distortion of the arrangement of the chondrocytes. The vesicular cartilage zone was broad varying in breadth from 36 cells in the center to 65 cells at the periphery. Vascular invasion and cartilage destruction were occurring in a regular pattern.

Osteoblasts were very numerous and were of the active cuboidal type (Figure 42). Numerous large multinucleate syncytial cells were scattered throughout intertrabecular reticular connective tissue and along the margins of the trabeculae. Bone marrow activity was normal. Thin layers of osteoid tissue were deposited on the surface of the trabeculae. Thick layers of osteoid tissue were present along the periosteal surface of the cortex. The periosteum was composed of numerous active osteoblasts and fibroblasts (Figures 63, 64 and 65). Numerous syncytial cells were observed in the periosteal tissue where active remodeling of the bone was occurring and where mechanical stress was present from muscular attachments. Sharp small irregularly shaped trabeculae were arranged along the periosteal surface of the cortex which suggested the presence of micorfractures and the formation of exostosis.

Pig 2(3), Age, 63 days: Microscopic examination of the costochondral junctions of this animal revealed the same bone and cartilage structure as described for the preceding pig, number 1(3).

Pig 3(3), Age, 16 days: The resting and proliferating cartilage zones were normal in appearance. The zone of vesicular cartilage was narrow and averaged 14 cells in breadth. Distortion of the normal columnar arrangement of the zone of vesicular cartilage resulted from the irregular size and shape

of the chondrocytes. The pattern of vascular invasion and cartilage destruction was regular. Blood vessels were actively congested. Osteoblast activity was suppressed which resulted in deficient deposition of bone. The primary spongiosa consisted of osteochondral trabeculae having irregular sizes and shapes. Thin layers of osteoid tissue were deposited on these trabeculae. A few large irregularly shaped osteochondral trabeculae formed the secondary spongiosa (Figure 58). Blood vessels were actively congested and contained a large number of erythrocytes. The rate of growth of the bone marrow was suppressed. The cortex was thin. The periosteum was composed of a few fibroblasts and inactive osteoblasts of the squamous type.

Pig 4(3), Age, 10 days: The resting and proliferating zones of cartilage were normal in appearance. The zone of vesicular cartilage was broad and showed an irregular pattern of vascular invasion and cartilage destruction (Figures 27 and 32). The cell columns varied from 80 to 120 cells in breadth. Invading blood vessels were actively congested and contained large numbers of erythrocytes. Large foci of cartilage in the vesicular zone were undergoing serous degeneration and necrosis. The cartilage matrix had become homogenous and eosinophilic. A few osteochondral trabeculae of various sizes and having irregular shapes formed the primary spongiosa. The activity of the osteoblasts was suppressed

(Figure 44). Numerous multinucleate syncytial cells were present throughout the bone and metaphysis. Bone deposition was deficient. The secondary spongiosa was composed of numerous thick irregularly shaped osteochondral trabeculae (Figure 56). Osteoid layers of various thicknesses were deposited on the surface of these trabeculae (Figure 57). The intertrabecular reticular connective tissue was edematous. Blood vessels were actively congested. The rate of bone marrow growth was suppressed. The cortex consisted of thick irregularly shaped trabeculae. The periosteum had slightly increased numbers of osteoblasts and fibroblasts.

Pig 1(7), Age, 31 days: The microscopic structure of the costochondral junction of this pig was the same as described previously for pig 1(3) on page 75.

Pig 2(7), Age, 6 days: The resting and proliferating zones of cartilage were normal in appearance. The zone of vesicular cartilage was very broad and averaged 94 cells in breadth. Columns in the vesicular cartilage zone were twisted and distorted as the result of irregularities in the size and shape of the chondrocytes. The amount of intercellular cartilage matrix was increased. The pattern of vascular invasion and cartilage destruction was irregular. Foci of serous degeneration and necrosis occurred in the zone of vesicular cartilage (Figure 40). Several foci of vascular invasion were observed near the proximal margin of the vesicu-

lar zone. The primary spongiosa was composed of a few irregular, thick, osteochondral trabeculae. These trabeculae were distorted, twisted and compressed. The rate of osteoblastic activity was suppressed. The osteoblasts were predominately the inactive squamous type. Slight deposition of bone was occurring. A few multinucleate syncytial cells were present.

The rate of bone marrow growth was suppressed. The blood vessels contained few erythrocytes. A few scattered thin trabeculae having thin layers of osteoid tissue deposited on their surface fromed the secondary spongiosa. The cortex was composed of long thin trabeculae. The periosteum was thin and composed of a few inactive squamous type osteoblasts and a few fibroblasts.

Pig 3(7), Age, 42 days: The resting zone of cartilage was normal in appearance. Slight distortion of the cartilage columns and increased amounts of intercellular cartilage matrix occurred in the zone of proliferating cartilage. The zone of vesicular cartilage was broad and varied in breadth from 48 cells in the center to 64 cells at the periphery (Figure 34). The pattern of vascular invasion and cartilage destruction was regular (Figure 35). Osteoblasts were numerous and of the active cuboidal type (Figure 48). The blood vessels were actively congested. Mineralization was occurring

normally. The primary spongiosa consisted of long osteochondral trabeculae enclosed by thin layers of osteoid and numerous osteoblasts. These trabeculae contained a central core of mineralized cartilage matrix. A few syncytial cells were scattered in the intertrabecular connective tissues and along edges of the trabeculae. The secondary spongiosa consisted of long regularly shaped wide trabeculae. Numerous osteoblasts and syncytial cells were present. The bone marrow was normal in appearance. The cortex was thin. The periosteum was composed of active cuboidal osteoblasts and fibroblasts and contained numerous multinucleate syncytial cells (Figure 60). Thin layers of osteoid tissue were deposited on the periosteal surface of the cortical trabeculae (Figures 50 and 51).

Pig 4(7), Age, 13 days: The resting and proliferating zones of cartilage were normal. The zone of vesicular cartilage zone was narrow and averaged 15 cells. The pattern of vascular invasion was regular. Osteoblasts were active cuboidal type. The primary spongiosa consisted of a lattice of irregularly shaped, thin, osteochondral trabeculae which contained numerous foci of necrotic cartilage and many individual chondrocytes in thin layers. Osteoid tissue was present on the surface of the trabeculae or was absent. The blood vessels were actively congested. The intertrabecular spaces were filled with delicate reticular connective tissue. A few

scattered multinucleate syncytial cells were present. Numerous thin short osteochondral trabeculae formed the secondary spongiosa.

The rate of bone marrow growth was suppressed. The cortex consisted of a few thin trabeculae. The periosteum was thin and showed a slightly increased number of osteoblasts and fibroblasts.

Pig 1(9), Age, 20 days: The zone of resting cartilage was normal in appearance. Distortion of the cartilage columns and increased amounts of intercellular cartilage matrix were observed in the zone of proliferating cartilage. The vesicular zone was very irregular and ranged from 4 to 93 cells in breadth. Numerous foci of necrotic cartilage having a homogeneous eosinophilic matrix were present. The pattern of vascular invasion and cartilage destruction was irregular. The rate of osteoblastic activity was suppressed. Deficient amounts of bone was being deposited. The blood vessels were passively congested. A serous inflammation was present in the metaphysis (Figure 45). The intertrabecular reticular connective tissue was dense and edematous. Mineralization was normal in appearance. A few scattered large irregularly sized and shaped osteochondral trabeculae formed both the primary and secondary spongiosa. These trabeculae contained many necrotic and mineralized cartilage cells. Osteoid tissue oc-

curred in thin layers on the surface of the trabeculae or was absent. The blood vessels were passively congested. The rate of bone marrow growth was suppressed. The cortex was thin. The periosteum was composed of a few inactive squamous type osteoblasts and fibroblasts.

Pig 2(9), Age, 13 days: The resting and proliferating zones of cartilage were normal in appearance. The zone of vesicular cartilage was broad and varied from 20 to 40 cells in breadth. Vascular invasion and cartilage destruction was occurring in an irregular pattern. Osteoblasts were few in number. Their activity was suppressed (Figure 49). The blood vessels contained few erythrocytes. Mineralization was normal. The primary spongiosa consisted of an irregular lattice of osteochondral trabeculae. Osteoid tissue was either deposited in thin layers on the surface of the trabeculae or was absent. A few multinucleate syncytial cells were scattered throughout the metaphysis and diaphysis. The secondary spongiosa consisted of a few scattered irregularly shaped and sized trabeculae. The rate of bone marrow growth was suppressed. The cortex was formed by trabeculae having irregular shapes and sizes. The periosteum was thin and was composed of a few osteoblasts and fibroblasts.

Pig 3(9), Age, 14 days: The zones of resting and proliferating cartilage were normal in appearance. The zone of vesicular cartilage was narrow and irregular in breadth. This

breadth varied from 12 to 30 cells. The pattern of vascular invasion and cartilage destruction was irregular. The blood vessels were passively congested. Mineralization was occurring in a normal pattern. A lattice of a few long thin osteochondral trabeculae formed the primary spongiosa (Figure 38). These trabeculae consisted of a central core of mineralized cartilage matrix enclosed by a thin layer of bone or osteoid. Osteoblasts were few in numbers and of the inactive squamous type. The deposition of bone was deficient in amount. The intertrabecular spaces were filled with edematous delicate reticular connective tissue. The secondary spongiosa was long and thin composed of osteochondral trabeculae with thin bone or osteoid margins. The rate of bone marrow growth was suppressed. The blood vessels were passively congested. The cortex consisted of thin trabeculae of varying lengths. The periosteum was thin and was composed of inactive osteoblasts and fibroblasts.

Pig 4(9), Age, 15 days: The zones of resting and proliferating cartilage were normal in appearance. The zone of vesicular cartilage was very irregular in breadth and varied from 5 to 60 cells. The pattern of vascular invasion and cartilage destruction was irregular. Osteoblasts were numerous and of the cuboidal type. Blood vessels were actively congested. Mineralization was normal in appearance. Numerous multinucleate syncytial cells were present. The primary

spongiosa was composed of irregularly sized and shaped osteochondral trabeculae. These trabeculae contained many entrapped cartilage cells. Osteoid tissue was either deposited in thin layers on the surface of the trabeculae or was absent. Numerous small irregularly shaped osteochondral trabeculae formed the secondary spongiosa. The blood vessels were actively congested. Bone marrow growth was suppressed. The cortex was composed of a few thin trabeculae having irregular shapes and lengths. The periosteum had inactive squamous type of osteoblasts and had a few fibroblasts.

Pig 5(9), Age, 12 days: The zones of resting and proliferating cartilage were normal in appearance. The zone of vesicular cartilage was very narrow and varied from 1 to 6 cells in breadth (Figure 36). A few projections of 16 to 20 cells were present. The pattern of vascular invasion and cartilage destruction was regular. A single small focus of necrotic cartilage was found in the zone of proliferating cartilage (Figure 41). Mineralization was normal in appearance. The primary spongiosa consisted of a few long thin osteochondral trabeculae. These trabeculae were irregular in shape and were slightly distorted. Osteoid tissue was either deposited in thin layers on the surface of the trabeculae or was absent. Osteoblasts were few in numbers and of the inactive squamous type. Very few multinucleate syncytial cells were present. The blood vessels were passively congested. A

few irregularly shaped and sized trabeculae formed the secondary spongiosa and cortex. Bone marrow growth was retarded. The periosteum was composed of a few inactive squamous types of osteoblasts and a few fibroblasts.

Pig 6(9), Age, 13 days: The zones of resting and proliferating cartilage were normal in appearance. The zone of vesicular cartilage was narrow and varied in breadth from 5 to 15 cells. The pattern of vascular invasion and cartilage destruction was regular. The blood vessels were passively congested. Osteoblasts were present in moderate numbers and of the active cuboidal type. Mineralization was occurring in a normal rate. The primary spongiosa consisted of a very few irregularly shaped osteochondral trabeculae. Thin layers of osteoid tissue were deposited on the surface of the trabeculae. The secondary spongiosa and cortex consisted of a few long thin osseous trabeculae. The rate of bone marrow growth was suppressed. The periosteum was thin and composed of a few inactive squamous type osteoblasts and a few fibroblasts.

Microscopic examination of tissues at the proximal end of the tibia and the distal end of the femur from rats fed a commercial ration for laboratory rats resulted in the following observations.

Rat (N-33), Age, 55 days: The proliferating zone of cartilage was narrow. The cartilage columns were uniform in

size and shape and were straight. They averaged 15 cells in breadth. The zone of vesicular cartilage was narrow and averaged 6 cells in breadth. The cartilage columns were straight and uniform in arrangement (Figure 66). Vascular invasion was in a regular pattern. The primary spongiosa consisted of thin interlacing osteochondral trabeculae. Osteoblasts were numerous and of the active cuboidal type. A few multinucleate syncytial cells were seen at the point of vascular invasion and cartilage destruction. The blood vessels were actively congested. The secondary spongiosa was formed by thick trabeculae. These trabeculae contained small central cores of cartilage and had thick layers of bone deposited on their surface. Bone marrow activity was normal in appearance (Figure 85) and myeloid cells extended into the intertrabecular spaces of the primary spongiosa. The cortex was thick and was composed of nearly solid bone with haversion canals (Figure 83). The periosteum was thin but had active cuboidal osteoblasts and numerous fibroblasts. A few syncytial cells were present in the periosteum. Osteoid tissue was not observed. Mineralization was normal in appearance.

Rat (N-71), Age, 93 days: Microscopic examination of tissues from this animal revealed the same bone and cartilage arrangement as described for the preceding rat (N-33). The only differences were in the maturity of the bone. The car-

tilage columns were less uniform and greater amounts of intercellular cartilage matrix were present in the proliferating and vesicular zones (Figure 67). The cortex was thicker and denser.

Microscopic examination of tissues from the proximal end of the tibia and distal end of the femur from rats fed a standard ricketogenic diet, U.S.P. Rachitogenic Diet No. 2, resulted in the following observations.

Rat (R-31), age, 53 days: The zone of proliferating cartilage was broad and contained uniform straight columns of cells. These cartilage columns averaged 23 cells in breadth. The zone of vesicular cartilage zone was irregular in breadth and varied from 7 to 16 cells (Figures 70 and 71). Projecting "tongues" of the vesicular cartilage columns, from 84 to 108 cells in breadth, extended into the diaphysis region of the metaphysis. The cartilage columns in these projections were compressed and twisted (Figures 74 and 75). This distortion was most severe in the areas of greatest mechanical stress.

The primary spongiosa was composed of a broad zone of thick osteoid trabeculae. This broad zone of osteoid tissue is sometimes termed the rachitic metaphysis. These trabeculae contained numerous cartilage cells embedded in the osteoid. The blood vessels were actively congested and branched extensively in a bush-like arrangement (Figure 72). Osteoblasts were numerous and most active in a narrow band along zones of

vascular invasion and cartilage destruction (Figure 76).

The secondary spongiosa consisted of a few scattered thick osteoid trabeculae which contained thin central cores of bone. Bone marrow activity was normal in appearance. The cortex was thick and consisted of almost solid osteoid tissue (Figure 79 and 80). The periosteum was thin and had a few active cuboidal shaped osteoblasts distributed along the cortical border.

Rat (R-41), Age, 63 days: The proliferating zone of cartilage was very broad. The increased amounts of intercellular cartilage matrix and the irregularities in the size and shape of the chondrocytes resulted in distortion of the cartilage columns. The zone of vesicular cartilage zone was very irregular in shape and varied from 12 to 26 cells in breadth. Projecting "tongues" of cartilage columns, 70 cells in breadth, extended into the metaphysis. Large focal areas of necrotic cartilage were seen along the distal epiphyseal border (Figure 73). At the edges of foci of necrotic cartilage, individual chondrocytes were showing rejuvenation and multiplication had formed small nests of cartilage cells nests. Areas of cartilage were undergoing metaplasia and forming pseudo-osteoid. The primary spongiosa consisted of a narrow zone of thick osteoid trabeculae which contained entrapped chondrocytes. Osteoblasts were numerous and of the

active cuboidal type. The blood vessels were actively congested and branched in bush-like patterns. The spongiosa was composed of a few irregularly shaped osteoid trabeculae. Small central cores of cartilage were contained within these trabeculae. Bone marrow growth was active. The cortex was thick and was composed principally of osteoid. The periosteum was thin and consisted primarily of a thin sheet of inactive osteoblasts and fibroblasts.

Rat (R-71), Age, 93 days: The proliferating zone of cartilage averaged 14 cells in breadth. Distortion of the cartilage columns resulted from large amounts of intercellular cartilage matrix. Transition between the zones of proliferating and vesicular cartilage was indistinct. The zone of vesicular cartilage was very irregular ranging from 10 to 65 cells in breadth. The pattern of vascular invasion and cartilage destruction was irregular. Irregular foci of blood vessels surrounded with osteoid tissue were scattered throughout the zone of vesicular cartilage. Irregular projecting "tongues" of vesicular cartilage extended into the diaphysis. Numerous active cuboidal shaped osteoblasts lined the blood vessels along the proximal edge of the metaphysis. The blood vessels were actively congested and bush-like in arrangement. The primary spongiosa was composed of thick osteoid trabeculae. Scattered irregularly shaped osteoid trabeculae formed the

secondary spongiosa. Bone marrow activity was normal in appearance. The cortex was thick, while the periosteum was thin.

Microscopic examination of tissues at the proximal end of the tibia and the distal end of the femur, from rats fed the U.S.P. Vitamin A Test Diet resulted in the following observations.

Rat (A-47), Age, 69 days: The zone of proliferating cartilage averaged 11 cells in breadth. Cartilage columns were slightly twisted and contained increased amounts of intercellular cartilage matrix. The zone of vesicular cartilage averaged 9 cells in breadth (Figure 69). Osteoblasts were few and were of the inactive squamous type (Figure 77). A few trabeculae having irregular sizes and shapes formed the primary spongiosa. The blood vessels were actively congested. The intertrabecular spaces were edematous and contained small amounts of reticular connective tissue. Mineralization was normal in appearance. Osteoid tissue was absent. A few scattered dense trabeculae formed the secondary spongiosa. The rate of bone marrow growth was severely suppressed (Figure 86). Numerous mast cells were scattered throughout the marrow tissues. The cortex was thin and dense (Figure 84). The periosteum was thin and was composed of inactive squamous type of osteoblasts and a few fibroblasts.

Microscopic examination of tissues at the proximal end of the tibia and the distal end of the femur from rats fed the experimental synthetic milk diet, Ration A, resulted in the following observations.

Rat (M-31), Age, 53 days: The zone of proliferating cartilage was broad. Cartilage columns were uniform, straight and averaged 18 cells in breadth. The vesicular zone of cartilage was narrow. It averaged 6 cells in breadth. Chondrocytes were regular in size and had matured in a uniform progression. The pattern of vascular invasion and cartilage destruction was regular. Osteoblasts were numerous and of the active cuboidal type. Blood vessels were actively congested. Mineralization was normal in appearance. A network of numerous small osteochondral trabeculae formed the primary spongiosa. Thick layers of osseous tissue were deposited on the surface of these trabeculae. Osteoid tissue was absent. The bone marrow activity was normal in appearance. The cortex was thick and the periosteum was thin and was composed of inactive squamous type of osteoblasts and fibroblasts.

Rat (M-42), Age, 64 days: The zone of proliferating cartilage was broad and averaged 18 cells. Increased amounts of intercellular cartilage matrix present with slight twisting of the cartilage columns. The zone of vesicular cartilage averaged 6 cells in breadth. Vascular invasion and cartilage destruction was occurring in a uniform pattern. Osteoblasts

were numerous and of the active cuboidal type (Figure 78). The blood vessels were actively congested. The primary spongiosa was composed of a few straight osteochondral trabeculae. Osteoid tissue was absent. Mineralization occurred in a normal pattern. Thick trabeculae formed the secondary spongiosa. The bone marrow activity was normal in appearance. The cortex was thick and dense (Figure 81). The periosteum was thin and was composed of a few inactive squamous type osteoblasts and a few fibroblasts.

Rat (M-71), Age, 93 days: The zone of proliferating cartilage averaged 12 cells in breadth. The cartilage columns were twisted and contained increased amounts of intercellular cartilage matrix. The vesicular zone averaged four cells in breadth. Vascular invasion was occurring in a uniform pattern. Thin osteochondral trabeculae formed the primary spongiosa. Osteoblasts were numerous and of the active cuboidal type. Mineralization was normal in appearance. Thick osseous layers enclosed the trabeculae. The bone marrow activity was normal in appearance. The cortex was thick and dense (Figure 82). The periosteum was thin and was composed of inactive squamous type of osteoblasts and a few fibroblasts.

Microscopic examination of tissues at the distal end of the femur and the proximal end of the tibiotarsus from chicks fed the control diet, U.S.P. Rachitogenic Diet No. 2 resulted in the following observations.

Chick (R-12), age 13 days: The resting and proliferating zones of cartilage were narrow. The zone of vesicular cartilage was broad and contained many large vascular channels which extended from the metaphysis to the zone of proliferating cartilage. These vascular channels were lined by a thick layer of osteoid. Osteoblasts were few and primarily of the inactive squamous cell type. The metaphysis was composed of a few small thin osteochondral trabeculae. The growth of bone marrow was suppressed. The cortex was thin. The periosteum was thin and was composed of inactive squamous type osteoblasts and a few fibroblasts.

Chick (R-17), Age 18 days: The resting and proliferating zones of cartilage were narrow. The zone of vesicular cartilage was very broad. Vascular invasion and cartilage destruction was irregular in pattern and suppressed. Focal areas of thick osteoid tissue were present in the metaphysis. Most of this osteoid tissue was deposited on the surface of the vascular channels. Osteoblasts were numerous and of the active cuboidal type. The intertrabecular spaces were filled with reticular connective tissue. Bone marrow activity was near normal. The cortex was thin. The periosteum was thin and was composed of inactive squamous type of osteoblasts and a few fibroblasts.

Chick (R-19), Age, 20 days: The resting and proliferating zones of cartilage were narrow. The zone of vesicular car-

tilage zone was very broad and contained numerous large vascular channels. These channels were bordered by thick layers of osteoid tissue, numerous active cuboidal shaped osteoblasts and congested blood vessels. Irregularly sized osteochondral and osteoid trabeculae formed the metaphysis. Bone marrow activity was normal. Numerous multinucleate syncytial cells were scattered throughout the intertrabecular spaces. The cortex was thick with wide osteoid borders. The periosteum was thick and was composed of active cuboidal shaped osteoblasts and numerous fibroblasts.

Chick (R-27), Age, 28 days: The resting and proliferating zones of cartilage were narrow. The zone of vesicular cartilage was very broad with a few long vascular channels. Osteoblasts were numerous and of the active cuboidal type. The blood vessels were actively congested. Thick layers of osteoid tissue bordered the trabeculae and vascular channels (Figure 87). Several large foci of necrotic cartilage were present in the distal regions of the metaphysis. The metaphysis consisted of a few irregularly shaped thin osteochondral trabeculae covered by varying amounts of osteoid. Bone marrow activity was suppressed. The cortex was thin with thick osteoid layers on periosteal surface. The periosteum was thin and was composed of inactive squamous type osteoblasts and a few fibroblasts.

Chick (R-57), Age, 58 days: The resting and proliferating

zones of cartilage were narrow. The zone of vesicular cartilage was very broad with numerous long narrow vascular channels. These vascular channels were lined by a thick layer of osteoid and numerous active cuboidal shaped osteoblasts (Figure 91). The cartilage in the distal portion of the vesicular zone stained intensely with the hematoxylin dye. The cartilage trabeculae of the vesicular zone were compressed and twisted (Figure 90). Many areas evidenced metaplasia into pseudo-osteoid. Foci of necrotic cartilage were present. The blood vessels were actively congested. A network of osteochondral trabeculae formed the metaphysis. These trabeculae were of irregular shapes and sizes and contained centrally located cores of cartilage. Thick layers of osteoid tissue were deposited on the surface of the trabeculae. Bone marrow activity was suppressed. The cortex was thick. A few multinucleate syncytial cells were present. The periosteum was thick and was composed of active cuboidal shaped osteoblasts and numerous fibroblasts. Thick layers of osteoid tissue were deposited along the periosteal surface of the cortex (Figure 89).

Microscopic examination of tissues at the proximal end of the tibiotarsus and the distal end of the femur from chicks fed the experimental synthetic milk diet, Ration A, resulted in the following observations.

Chick (M-19), Age, 20 days: The resting and proliferating zones of cartilage were narrow. The zone of vesicular cartilage was broad. Long vascular channels penetrated the zone of vesicular cartilage from the metaphysis and extended into the zone of proliferating cartilage. Thick layers of osteoid tissue were deposited on the surface of the vascular channels in the zone of vesicular cartilage and the surface of the osteochondral trabeculae in the metaphysis (Figure 88). Osteoblasts were numerous and of the active cuboidal type (Figure 92). Numerous multinucleate syncytial cells were present. The intertrabecular spaces were filled with dense reticular connective tissue (Figure 93). Bone marrow activity was normal. The cortex was thin. The periosteum was thin, was composed of increased numbers of osteoblasts and fibroblasts and contained many multinucleate syncytial cells. These syncytial cells were most numerous in the cortical area adjacent to the epiphyseal-diaphyseal junction.

Chick (M-27), Age, 28 days: The resting and proliferating zones of cartilage were narrow. The vesicular zone of cartilage was narrow and contained numerous wide, short vascular channels. Numerous thin osteochondral trabeculae formed the metaphysis. Osteoblasts were numerous and of the active cuboidal type. Numerous multinucleate syncytial cells were scattered throughout the intertrabecular spaces (Figure 94). The blood vessels were actively congested. Thin layers of

osteoid tissue was deposited on the surface of the vascular channels and trabeculae. Bone marrow activity was suppressed. The cortex was thick and had wide layers of osteoid tissue on the periosteal surface. The periosteum was thin and was composed of increased numbers of osteoblasts and fibroblasts. Many syncytial cells were present along the cortical border of the periosteum.

Chick (M-57), Age, 58 days: The resting and proliferating zones of cartilage were narrow. The zone of vesicular cartilage was narrow and very irregular as a result of the penetration of numerous large branching vascular channels. Osteoblasts were numerous and of the active cuboidal type. Thin layers of osteoid tissues were deposited on the surface of the vascular channels. A few irregularly shaped and sized trabeculae formed the metaphysis. Bone marrow activity was normal. The cortex was thin with a wide layer of osteoid tissue deposited on the periosteal surface. The periosteum was thick, fibrous and had numerous multinucleate syncytial cells. There was a mild periostitis near the epiphyseal-diaphyseal junction.

DISCUSSION

Hart and Steenbock (57), Dunlop (26), Braude et al. (12), Stolte (151), and Wahlstrom and Stolte (161) reported D vitamins were not required to prevent rickets in pigs on well-balanced rations composed of cereal grains, protein supplements and ample amounts of calcium and phosphorus in a favorable ratio.

These reports are contrary to those of Zilva et al. (169), Sheeny and Senior (132), Johnson and Palmer (75) and Bethke et al. (7) which describe definite D vitamin requirements to prevent rickets when adequate amounts of calcium and phosphorus were present in the ration.

This conflict in the conclusions by these two groups of investigators can be explained theoretically on the basis of differences in pre-experimental D-vitamin stores, ages, sunlight exposure, growth rates, length of experiments and individual pigs. However, controlled experiments are needed to establish the exact role of these variables in the production of rickets in swine.

During the course of this experiment, an unknown factor existed which may have interfered with this investigation of classical rickets. It is possible this factor is a characteristic of an uncomplicated D vitamin deficiency in baby pigs

on a synthetic milk diet. However, in the review of the literature on rickets and D vitamin deficiencies, no descriptions were recorded of the severe retardation of growth (100 percent), generalized edema (75 percent) and deaths (85 percent) which occurred prior to the fourth week of this experiment. The administration of injectable D vitamins did not prevent or alleviate this syndrome.

Retarded growth was expected to a moderate extent because of preceding experiments by other investigators both as a specific result of the D vitamin deficiency (126, 104) and the non-specific effect of early weaning to an artificial diet (30, 153). However, the retardation of growth was quite severe in this experiment as demonstrated by the weight of 6 pounds by pig 1(3) and the weight of 11 pounds by pig 2(3) at 46 days of age. The expected average weight for a pig of this age is 36 pounds (145, 154).

On the 28th day of the experiment, pig 2(3) began to recover from the inhibitory effect of the experimental diet and evidenced an increased growth rate. His weight at 46 days had doubled that of the 28th day. At the termination of this experiment on the 63rd day, he weighed 27 pounds.

In the case of pig 1(3), an increased growth rate did not become apparent until the 56th day. At this time, he

began to grow rapidly and had almost tripled his weight by the end of the experiment on the 63rd day. Nevertheless, it must be noted his 17 pounds is far short of the expected average weight of 45 pounds at nine weeks (145, 154).

As reported previously in the literature review, growth is essential for the production of rickets in either its classical or juvenile forms. The failure of the pigs to grow as rapidly as anticipated, may have been a major factor in this investigation. Any future experiments in the study of rickets must provide for maximum growth to eliminate this factor. It should be noted that microscopically pigs 1(3), 2(3), 1(7) and 3(7) evidenced a normal growth pattern which suggests that sufficient growth was present for development of rickets in these animals after the third week.

Subcutaneous edema and death were not expected results of this experiment. Because the primary object of this experiment was to study the bone changes in these pigs, limited funds, personnel and facilities prevented a detailed study of this edema. Nevertheless, a general discussion of this edema is presented for consideration in future studies.

Edema is a condition in which excessive amounts of fluid occur in the intercellular spaces or body cavities (127, 142). It may result from injury to the capillary wall which

increases its permeability such as caused by a variety of toxic agents, endotheliotropic viruses and hypoxemia. Edema may be caused from increased capillary blood pressure as caused by venous obstructions or cardiac insufficiency. Edema may result from a decrease in colloid osmotic pressure caused by decreased plasma protein levels as occurs in chronic anemia, starvation and cachetic diseases. Edema also may result from lymphatic obstruction, allergic reactions, hormonal imbalances and neurovascular disturbances. Edema may be either local or general in distribution. Subcutaneous edema may also be termed anasarca.

The pigs used in this experiment were selected at random from different litters, separated from healthy sows six to eighteen hours after birth and raised in individual units isolated from each other and other swine. These precautions were used to minimize the possible presence of a contagious or hereditary disease.

The rations fed were well-balanced, designed specifically for baby pigs and fortified with vitamins and trace minerals. They contained high amounts of B vitamins, ascorbic acid and both animal and vegetable proteins. The only known factor lacking in these diets was D vitamins. It should be noted that the skim milk used in this experiment may have contained minute amounts of D vitamins (104). However, the amount pres-

ent is far short of the requirements expected for baby pigs at this age (31, 169). Studies in classical rickets have shown the minute amounts of D vitamins in human milk and cows milk were inadequate to prevent rickets (64, 67, 114). Experimental studies in puppies (99, 100), rats (9) and chicks (59) support this research in the human infant.

Clinical examination revealed the pigs first become either excitable or listless and incoordinated followed by depression and weakness. When this weakness became severe, the pigs were unable to stand or eat. Death occurred six to twelve hours after the severe weakness developed. This clinical syndrome is suggestive of an intoxication or toxemia. Since toxins are a common cause of edema in older swine, the possibility that a toxemia was the primary agent in this edema must be considered.

Edema disease of swine is a specific disease of this genus characterized by the presence of extensive amounts of edema in various tissues of the body. Bennett (5) reports this edema is present in the stomach wall, colonic mesentery, eyelids, ears, subcutaneous tissues of the face and joints, and the ventral and ventrolateral areas of the abdominal wall. He bases his diagnoses primarily upon the clinical history and post mortem examination. He regards edema disease as a toxemia of possible bacterial origin. No attempts were made

to demonstrate bacterial toxins in these animals. Because of the absence of known toxins in these cases, it is possible this disease is a syndrome resulting from several causative agents.

Underdahl et al. (158) report the transmission of an agent which produces the clinical signs and pathologic changes associated with edema disease. Their agent is suggested to be a virus. Because this is a preliminary report additional study is in progress to evaluate and characterize the agent isolated.

Experimentally Gregory (47) has produced a severe subcutaneous edema in mice using a specific toxin from beta hemolytic Escherichia coli strains. In naturally occurring cases, Gelenczei (45) and Underdahl et al. (158) report the same lesions as described by Bennett (5) as the result of toxins produced by this same bacterial organism. It must be noted that in both the experimental and spontaneous cases of edema disease, edema is not a consistent lesion in either location or severity.

In this experiment, an extensive subcutaneous edema was consistent in these pigs. The only variation was the severity of the lesions in specific areas.

Extensive subcutaneous edema accompanied by hemorrhage

occurs in the horse (127 and 157). This condition is called purpura hemorrhagica and occurs following bacterial infections. Purpura hemorrhagica is believed the result of toxic injury to the capillary endothelium caused by bacterial toxins in the circulation. The specific mechanism has been suggested to be an anaphylactoid reaction associated with a possible thrombocytopenia.

In the human being, a condition of subcutaneous edema is sometimes associated with delayed allergic reactions such as serum sickness (14, 125, 134). Serum sickness is usually regarded as a type of anaphylactic shock caused by a sensitivity to substances in horse or rabbit antiserum. In addition, it has been shown that many other foreign proteins and drugs, such as purified toxoids, penicillin, sulfonamides, and procaine hydrochloride, may produce similar reactions in sensitized individuals. This disease is characterized by the occurrence of symptoms about 8 to 10 days after the administration of the antigen and persists for several days. This interval from initial administration denotes the induction period for antibody formation and the hypersensitive state; if at the end of this period antigen still remains in the body, a reaction occurs which results in increased capillary permeability and edema of the subcutaneous tissues. Since the pigs on this experiment were being fed a foreign protein less

than twenty-four hours after birth, the possibility of an allergic angioedema is present.

External examination of these pigs revealed a rough hair coat. The skin was distended by fluid in the subcutaneous tissues of the ventral areas of the body. This distention was most pronounced in the submandibular region, ventral cervical and abdominal regions, thighs and hocks. In severe cases, the skin was thrown into distinct folds, (Figures 3, 5, 6 and 9). The ears were thick and rolled at their tips, (Figures 3 and 4). Post mortem examination revealed a severe extensive subcutaneous edema which also involved the skeletal connective tissues throughout the entire body (Figures 19 and 20). Many of the animals had increased amounts of pericardial, pleural and peritoneal fluids. The livers, kidneys, hearts, and spleens were normal in appearance. The lungs were usually congested with atelectatic ventral borders on the apical and cardiac lobes. Bronchopneumonias were evidenced by two animals, 5(30) and 3(7). The blood was frequently a dark reddish brown as a result of a terminal circulatory failure. There was no evidence of anemia or bacterial septicemias.

Subcutaneous edema has been described in man and animals as a result of generalized passive hyperemia caused by cardiac insufficiency (1, 11, 127, 142). In this experiment, the

hearts appeared normal and no evidence of a general passive hyperemia was present.

Another common cause of subcutaneous edema in the human being is the hypoproteinemia following renal injury (11, 27, 142). This condition is rare in animals. There was no evidence of renal injury in the pigs on this experiment. However, additional study would be required to eliminate this possibility from consideration in the etiology of the extensive subcutaneous edema.

Hypoproteinemia resulting from either a primary amino acid deficiency or an inability to assimilate essential amino acids from the diet are additional possibilities to be considered (34, 127).

Cartwright et al. (15) using a synthetic diet containing the acid hydrolysate of casein or zein produced retarded growth, anemia, hypoproteinemia and edema. They thought these changes were most likely caused by a deficiency of tryptophan. Harris, H. A. et al. (54) produced hypoproteinemia in rats with a lysine deficiency. The ration fed in this experiment was largely composed of milk products high in both tryptophan and lysine content. In experiments by other investigators using diets similar to Ration A, no difficulties with subcutaneous edema were reported (42, 107, 164, 167, 168).

Extensive subcutaneous edema is seen frequently in ani-

mals which have a hypoproteinemia concurrent with an anemia resulting from continuous or reoccurring hemorrhage (127). This condition is usually associated with parasitic infections such as Haemonchus species in sheep and cattle. Chronic bleeding from gastric ulcers may also produce anemia and hypoproteinemia.

There was no evidence of anemia in the pigs in this experiment. However, future investigations of generalized edema should include hematological examinations to eliminate a possible latent anemia. No parasites were present.

In experiments using dogs, Markowitz et al. (88), describes the production of generalized experimental edema by plasmapheresis and by low protein diets. This edema occurred when the plasma protein level reached 3.5 percent. They observed that if the protein level in the serum was depleted gradually by low protein diets, the edema was more likely to occur in the subcutaneous tissues than in the peritoneal cavity. If the protein content was depleted rapidly by plasmapheresis, ascites was invariably present and edema of the extremities was less intense. The salt content of the diet was reported to be an additional factor in the production of subcutaneous edema. They observed that a mild edema could be transformed into general anasarca by ingestion of salt by a patient with hypoalbuminemia. They also reported experimental

edema could be eliminated by forced muscular activity. The confinement of the baby pigs to rabbit cages during this experiment prevented adequate exercise of these animals. Therefore, this is an additional factor in the severity of the edema observed. The diet contained a normal amount of salt.

In generalized edema, the role of the endocrine glands and the effect of disturbances in metabolism from non-infectious intrinsic factors is difficult to evaluate. However, these areas definitely have been shown to play an important role in electrolyte balances and proteins metabolism. Swenson and Talbot[1] produced severe generalized subcutaneous and interlobular pulmonary edema in pigs in advanced anemia by the injection of adrenal cortical hormones. Because this was a preliminary study, the mechanism by which this edema was produced is still unknown. However, these results indicate the adrenal cortical hormones and possibly stress may be important factors in the generalized edema which occurred in this experiment. Vesselinovitch (159) investigating the serum proteins of pigs affected with edema disease concluded that a non-specific dysproteinemia present in these cases may result from a non-specific reaction of the body to stress accompnaied by an enhanced activity of the reticulo-endothelial system.

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[1]Swenson, M. J. and Talbot, R. B. Iowa State University of Science and Technology, Department of Veterinary Physiology and Pharmacology, Ames, Iowa. Experimental edema in anemic pigs. Private communication. 1961.

Generalized subcutaneous edema as observed in these pigs is seldom seen as a natural occurring or spontaneous disease. However, during the last six months, seven cases have been observed in the Iowa Veterinary Medical Diagnostic Laboratory by this author (119). These cases were all in baby pigs being raised by Specific Pathogen Free swine breeders in isolation units and on synthetic diets similar to the procedures used in this experiment. In these cases, a wide variety of bacterial organisms were isolated including Escherichia coli, Protens species, beta hemolytic Streptococcus species, Bacillus species, Pseudomonas species and other unidentified coliform organisms.

This failure to isolate any consistent bacterial agent from these pigs suggests this condition is not the result of a toxin produced by a single species of bacteria. No attempts were made to isolate a toxic agent from these animals.

As revealed in the preceding discussion the extensive subcutaneous edema which occurred in this experiment is the result of a physiochemical vascular disturbance. The etiology of this disturbance can not be established without additional intensive investigation.

Eighty-five percent of the pigs in this experiment died before the end of the third week. Five of these deaths occurred during the first six days. Four of these five pigs

were severely dehydrated and died within ten minutes to three hours after being observed sick. Two of these pigs went into convulsions shortly after being fed and died. Only one pig of the five had the extensive subcutaneous edema which characterized the remainder of the experiment. Ten (50 percent) of the experimental pigs died between the tenth and sixteenth days. Eight of these pigs evidenced extensive subcutaneous edema. These animals became depressed, weak and incoordinate shortly before their deaths. Weak respiratory movements or a mild dyspnea frequently was observed shortly before their deaths. This respiratory disturbance resulted from increased fluids in the thoracic cavities and depression of respiratory activity. Two animals had a pleuritis resulting from bacterial infections. Two animals which died after the sixteenth day had severe edema of the subcutaneous tissues, Pig 1(9) which died on the twentieth day and Pig 1(7) which died on the thirty-first day.

The remaining three pigs 3(7), 1(3) and 2(3) recovered from the edematous condition and were euthanized on the forty-second, sixty-third and sixty-third days respectively. Pig 3(7) had a chronic proliferative bronchopneumonia at the time of its death which was believed to have resulted from a bacterial infection during the period of edema.

The etiology of the spontaneous deaths which occurred

during this experiment is unknown. However, since these deaths can be correlated directly to the presence of edema or dehydration, it is believed they are the result of the same physiochemical fluid imbalances which caused the subcutaneous edema previously discussed. Death is not described as a principal characteristic feature of either classical or juvenile rickets.

The retarded growth, rough haircoats and unthriftiness exhibited by these pigs during the first two weeks of this experiment are non-specific symptoms of malnutrition (105). These observations have limited value in evaluating the bone lesions present. These symptoms may have been the result of either deficiencies in D vitamins or the complicating unknown factor or both.

As described previously, classical rickets is an osteodystrophic disease of the infant characterized by distortion of the skeletal system. Four pigs 1(3), 2(3), 3(3) and 3(7) exhibited a clinical osteodystrophy. This osteodystrophy was manifest either by the hocks or the digits of the front feet being turned medially. Two pigs 1(3) and 2(3) had distortion of both the front and hind legs. Pig 2(3) was severely affected. The elbows were markedly bowed laterally at the thorax and the carpals were overextended. The stifle and metatarsal region rotated laterally with severe involvement of the

latter. The digits of the hindfeet were sharply turned medially causing the weight of the animal to rest on the sole of the lateral digits. This pig began growing rapidly near the termination of the experiment and was prevented from proper exercise by the small size of its quarters (Figure 12). Much of the time this pig laid on his abdomen with his hind legs extended anteriorly as demonstrated by Figure 15 .

In 1908, Findlay (33) concluded from his experiments with dogs that confinement with its consequent lack of exercise was a major factor in the development of rickets. When in 1921, Mellanby (99) demonstrated a fat soluble vitamin deficiency was the essential cause of rickets, he also demonstrated confinement and its lack of exercise was a contributing factor in producing the disease. In experiments of both investigators, dogs which did not exercise were more severely affected by rickets than those which did.

In addition to exercise, two other mechanical factors are important in the etiology of osteodystrophy. These are body weight and body conformation. Large active rapidly growing animals are more susceptible to bone disease than small animals of the same genus (10, 70). This is easily demonstrated in dogs where the large dog breeds such as German shepherds, are much more susceptible to osteodystrophy than fox terriers (68).

Body conformation and position are important in maintaining correct lines of stress, and tension for body support and proper functioning of joints (86). If body conformation is abnormal as a result of heredity or disease, or position because of environment, the bone undergoes a remodeling process in an attempt to re-establish functional support of the body and efficiency in movement. Therefore if an normal animal is maintained in an abnormal position for a long period of time, an osteodystrophy may result from environmental conditions. These bone lesions as in lameness will be intensified by faulty conformation and weak bone structure (41, 102).

The osteodystrophy of rickets results from the mechanical stresses of body weight and muscle tension upon soft unossified bone (11, 112). Ricketic bone is easily cut with a post mortem knife. The bones of the pigs in this experiment which exhibited osteodystrophies had normal dense well-ossified bones which were difficult to cut either with a jeweler's saw or a post mortem knife.

Gross osteodystrophy has been reported in swine on rations deficient in copper by Teague and Carpenter (152), Lahey et al. (83) and Follis et al. (37). Teague and Carpenter (152) report its appearance during the fourth week in all of the experimental pigs. The front legs were bowed laterally at elbows and turned medially at the distal ends. The hind legs were abnormally crooked and lacked rigidity.

The pictures which accompany this article closely resemble pig 2(3) in this experiment. Lahey et al. (83) report a similar condition in their experiments. Baxter and Van Wyk (3) report lesions in dogs which closely correspond to those described in swine. It should be noted in each of these experiments, the primary constituent of the diet was cows milk and all the animals were housed in the close confinement of cage units. Therapeutic use of copper prevented or alleviated this condition.

In addition to a copper deficiency, manganese deficiency in swine is reported by Miller et al. (103) and Neher et al. (106) to result in osteodystrophy. Miller et al. (103) report lameness, enlarged hock joints and crooked legs. This bone deformity became apparent only when the pigs reached a weight of 150 pounds. The incidence of osteodystrophy was 50 percent. Neher et al. (106) report a decrease in the length of the forelegs and hind legs, thickening of the carpal and tarsal areas and marked bowing of the front legs in 60 percent of the experimental animals. Similar osteodystrophies are reported in rats and rabbits placed on manganese deficient diets (91). Manganese supplementation prevented these deformities from developing. However, manganese was ineffective after the disease had developed. The exact role of manganese plays in bone growth is at present unknown.

Three pigs 2(7), 3(3) and 4(3) exhibited broad white lines at the costochondral junctions. Microscopical examination revealed these areas are broad mineralized vesicular cartilage zones undergoing irregular vascular invasion. In the normal pig, the ossified vesicular cartilage zone is observed as a narrow sharp white line.

Broad zones of ossified vesicular cartilage are frequently observed in pigs examined at the Iowa Veterinary Medical Diagnostic Laboratory. Groth (48, 49) describes in detail the histopathology observed in 48 of these cases. He suggests this osteodystrophy may be the result of hog cholera or swine influenza infections possibly accompanied by deficiencies in A vitamins or calcium. Dunne et al. (28) describe in detail the osteodystrophy of pigs infected with hog cholera. In subacute cases, the cartilage zone was greatly enlarged.

Locomotor disturbances are reported as essential symptoms of juvenile rickets in animals by many investigators, Smith and Jones (142), Hutyra et al. (70, 71), Kernkamp (79, 80) and Udall (157). These disturbances vary from a slight lameness to an inability to walk. The cause of this lameness has not been adequately described. It is stated fractures are frequent in affected animals which result in severe symptoms of a locomotor disturbance. Kernkamp (79, 80) and Marek and Wellmann (87) described degenerative changes in the articu-

lar cartilages associated with their experimental cases of juvenile rickets caused by rations low in calcium. The joints most frequently affected were the shoulder, elbow, carpus, hip, stifle and tarsus. Post mortem examination revealed degenerative cartilage lesions with wrinkling and furrowing of the articular cartilage and roughness or erosion of the articular surface. The joint capsule was thickened in some cases with villar hyperplasia. Hutyra et al. (71) report an arthritis may be present in addition to articular lesions. It is interesting to note these articular lesions closely resemble those described in the human being (86, 101), horses (13) and cattle (102) as osteoarthritis. The etiology of osteoarthritis is unknown. However, extensive study has revealed many of the contributing factors associated with this disease. Senility, trauma, skeletal deformities, intrinsic cartilage wearing ability and disturbances in the synovial membrane and synovial fluid are reported factors in degenerative changes of cartilage (86, 102).

Sampson et al. (128) report an osteodystrophy in swine characterized by unthriftiness, lameness and arthritis resulting from feeding excess zinc in the ration. The gross lesions primarily involved the articular surfaces with erosion, ulceration, folding and grooving of the cartilage surface. The joint capsules of the shoulder, elbow, hip and stifles

were abnormally distended and contained excessive synovial fluid. It is apparent from their discussion that an osteoarthritis was present in their pigs. Zinc metabolism has been shown to have close relationship to calcium metabolism. Therefore, it is probable the production of osteoarthritis by these two different diets is the result of the same basic mechanism. Additional investigation into the role of zinc and calcium in bone disease will be necessary to evaluate the significance of these osteoarthritis reports in swine.

Deficiencies in D vitamins have not been demonstrated to have a major role in osteoarthritis. During this experiment no lameness or joints lesions were observed. It should be noted these pigs were closely confined and were much younger than the pigs reported to have osteoarthritis.

Microscopic examination of the costochondral junctions of 14 out of 18 pigs in this experiment revealed a moderate to severe disturbance in endochondral bone growth. This disturbance was manifest in both the epiphyseal cartilage and the diaphysis.

The most significant lesions observed were variation in the breadth of the vesicular cartilage zone, irregularity in vascular invasion and cartilage destruction and suppression of osteoblastic function, with deficient bone deposition in

the metaphysis and cortex.

Bone and cartilage have been shown to be very sensitive to changes in the health status of the individual (34). As a result of the sensitivity, any condition which interferes with the normal physiological process of growth, the vascular system or nutrition is quickly reflected in the skeleton. This response may be specific or nonspecific depending upon the etiological agent and the exact cells affected. Most diseases affect both bone and cartilage. A few agents such as copper and ascorbic acid deficiencies, affect principally specialized cell types, the osteoblasts or undifferentiated endothelial cells.

The response of bone and cartilage to nutritional disturbance has been shown to be rapid. Experimental studies by Dodds and Cameron (22) indicate ricketic changes can be observed in rats after twenty-four hours on their ricketogenic diet. Steenbock and Black (146) and McCollum et al. (95) detected sufficient ossification in ricketic rats for satisfactory "line tests" for D vitamin activity--five days after the addition of D vitamins to the diet. Harris (53) reports experimental production of growth arrest (brachychondroplasia) in puppies after 72 hours of inanition.

In this experiment, extensive microscopic cartilage and bone changes were present in pigs after four days. Broad

white lines could be seen at the costochondral junctions at 6 days. These results confirm the rapid changes which occur in bone and skeletal growth as a result of nutritional or metabolic disease.

Normal bone and cartilage growth depends upon primarily the function of three cell types, the undifferentiated endothelial cells of the capillary bud, the specialized chondrocytes of the epiphysis and the specialized osteoblasts of the metaphysis and diaphysis. The endothelial cells of the capillary bud are essential in the process of cartilage invasion and destruction. They are also believed by some authors to give rise to the osteoblasts by differentiation (81). Ham (51) believes osteoblasts result by multiplication of osteoblasts. In either case, osteoblasts function to deposit osteoid which normally is quickly ossified to form functional bone. The chondrocytes arise primarily by multiplication of chondroblasts, in the resting and proliferating cartilage zones and form the epiphyseal plate essential in the endochondral bone growth of mammals.

In addition to endothelial cells of the capillary bud, the chondrocytes, and osteoblasts, many other cell types contribute to the process of bone and cartilage growth. Osteocytes maintain the bone matrix. Multinucleate syncytial cells (chondroclasts and osteoblasts) and mononuclear phagocytes function in bone and cartilage destruction (19). Fibro-

blasts and fibrocytes provide support for the bone marrow and blood vessels. Vascular endothelium forming the blood vessels carry essential nutrients to the tissues and remove metabolic products. The function of the bone marrow in bone growth is believed minimal.

In bone and cartilage diseases, the specific microscopic lesions depend upon the specific cell types affected. Some cells are more easily affected by metabolic disturbances and therefore are affected by different etiologic agents at different rates.

In classical rickets, the principal disturbance is a failure of ossification of osteoid (11). The osteoblasts continue to function resulting in broad osteoid zones and borders. Chondrocytes continue to grow and multiply but fail to mature. The intercellular cartilage matrix fails to mineralize. Vascular invasion and cartilage destruction cease.

In moderate or mild deficiencies of A vitamins, the endothelial cells of the capillary bud lose their ability to invade cartilage and fail to differentiate into osteoblasts and endothelium (48). Chondrocytes are only slightly affected and continue to grow and multiply resulting in broad cartilage plates. The absence of osteoblasts results in deficient bone deposition, thin trabaculae and thin cortices.

In severe deficiencies of A vitamins, the entire process of endochondral growth is retarded (49, 166). Chondrocytes fail to grow and multiply. Vascular invasion and cartilage destruction cease. Endothelial cells and osteoblasts fail to differentiate. Bone deposition ceases. Thin trabeculae and cortices result. Growth arrest has occurred.

In ascorbic acid deficiency, chondrocytic multiplication and growth continue at a normal rate (30). Vascular invasion and cartilage destruction proceed normally. A lattice of thin osteochondral trabeculae forms in the primary spongiosa as a result of suppressed osteoblast function with deficient deposition of bone. In classical scurvy, as a result of muscle tension and stress, these thin trabeculae fracture accompanied by extensive hemorrhage of injured blood vessels and organize to form the characteristic "Trummerfeld" and "Gerustmark" described by early German pathologists (35).

Calcium deficiency is reported to result in either rickets (juvenile) or osteomalacia combined with osteoporosis depending upon the age of the calcium-deficient individual (110, 157, 163). The microscopic changes of rickets and its effect on the epiphyseal cartilage have been previously described. In osteomalacia, the epiphyseal cartilages show little change because they have ceased to grow with maturity of the individual involved. The prominent lesions are thick osteoid

layers bordering the trabeculae and in the cortex. Osteoblastic function and vascular invasion are normal. Osteoporosis results from a deficiency in the deposition of osteoid and therefore bone (11). Osteoblastic function is suppressed.

Phosphorus deficiency in experimental animals produces lesions of rickets (34, 38, 155, 156). The microscopic lesions in rats are reported to be identical to those described in the human infant. The microscopic changes in the epiphyseal cartilage have been previously described.

Poisoning by metallic phosphorus, lead, arsenic and bismuth produces a disturbance of the ossified cartilage which results in thick trabeculae of cartilage (86, 110, 112). These thick cartilage are later ossified to form thick bone trabeculae. The thick bone trabeculae formed are seen on radiographs as rings or lines of increased density. Harris (53) terms these lines of arrest of growth. Weinmann and Sicher (163) designate these same lines as osteosclerotic bands. They suggest metallic phosphorus acts upon the intercellular cartilage matrix to form irritating non-absorbable bone. The surrounding osseous tissues undergo hyperplasia in an attempt to encapsulate the damaged bone. This hyperplasia results in the osteosclerotic bone.

Flourine poisoning is reported to result in either osteosclerosis, osteoporosis or osteomalacia, depending upon the

dosage used and the age of affected individual (138, 163), Weinmann and Sicher (163) indicate the basic mechanism is rapid absorption of bone which is accompanied by a compensatory osteophytic hyperplasia of the periosteal tissues. In young animals rapid absorption of the bone results in almost complete removal of the dense compact bone leaving only the newly formed hyperplastic bone. At lower doses and in older individuals, destruction of bone is slower and formation of hyperplastic bone is slower resulting in more compact and dense bone (osteosclerosis). Epiphyseal changes were not described.

Copper deficiency results in growth arrest lesions (4, 37). Follis et al. (37) report thin cortices, thin trabeculae, broad proliferating and vesicular cartilage zones, persistence of a lattice of osteochondral trabeculae with deficient bone deposition. These findings suggest the principal disturbance was in osteoblastic function.

Bone lesions in manganese deficiency in swine has been briefly described by Neher et al. (106). Histopathologically cancellous bone in the diaphysis of the ulna was replaced by a dense fibrous vascular connective tissue. The epiphyseal cartilage of the radiuses was thin and serrated in outline. Increased amounts of pale staining matrix was described adjacent to the proliferating cartilage zone.

Follis (34) indicates that rickets is the only disease which produces specific alterations in the growth of cartilage. He states that a lack of any one of the essential nutrients, water, carbohydrates, fats, proteins and amino acids, inorganic elements and vitamins, may result in a non-specific disturbance in the growth of cartilage.

Inanition, bacterial infections, viral infections, parasitic infections, and many metabolic diseases which have direct relationship to bone metabolism may also result in non-specific growth disturbances (16).

Cohen (16) describes two non-specific bone and cartilage disturbance forms, one form designated as "non-specific disturbance in growth", the other as "arrest of growth."

"Non-specific disturbance in growth" is characterized by an enlarged proliferating cell zone and a thickened vesicular cell zone with little evidence that the mature cells are being actively penetrated by the marrow vessels and connective tissues and slight deposition of bone. Cohen regards this variation in bone and cartilage growth as a temporary piling up of cartilage and a disturbance of the unknown mechanism by which it is converted into bone. The frequency with which this disturbance is observed in a variety of acute diseases suggests that it is non-specific.

Groth (48) studied microscopically the costochondral junctions of ribs of 48 Iowa swine. Microscopical examination revealed wide proliferating and vesicular cartilage zones, suppressed cartilage breakdown, thin trabeculae in the spongiosa, and aplasia or hypoplasia of the osteoblasts. These lesions closely resemble those described by Cohen. The animals used by Groth for his study were selected on the basis of gross lesions and represented a variety of etiological agents. Eleven pigs were diagnosed as hog cholera infections. Three pigs were known to be deficient in A vitamins. The other 35 pigs died from unreported causes but probably represented a group of miscellaneous diseases. It would have been valuable to have known additional information about these cases.

Because these bone and cartilage changes form a distinct pathological entity in the human being and swine, this writer proposes the "non-specific disturbance of growth" described by Cohen be termed "eurychondroplasia" (eurys = broad + chondro = cartilage + plassein = to form) (111) because of its characteristic broad cartilage. Eurychondroplasia is defined as a non-specific disturbance in endochondral bone growth and characterized by broad proliferating and vesicular cartilage zones, suppressed or irregular vascular invasion and cartilage destruction, thin trabeculae in the spongiosa and suppression of osteoblastic function with deficient bone deposition. Mineralization is normal. Osteoid is present only as thin

layers on the trabeculae and in the cortex.

"Arrest of growth" according to Cohen is usually seen in long-standing disease. The microscopical appearance is thin cartilage zones and suppression of vascular invasion. An incomplete thin band of osseous matrix is deposited at the distal ends of the vesicular columns which tend to seal off the cartilage columns. Study by Harris (53) indicates that arrest of growth is the secondary result of a variety of conditions and if the individual recovers from the primary condition, normal growth will return. In these cases, the lines of arrested growth are visible by radiographic examination for long periods, up to many years in the human being.

Arrested growth is produced in the human being by starvation, diabetes, bronchopneumonia, measles, whooping-cough, laryngitis, osteomyelitis, lymphatic leukemia, chicken-pox, influenza, "croup", tonsilitis, mumps, scarlet fever, and syphilis (53). Experimental study of rats with pneumonia, middle-ear infections and cerebellar abscesses exhibited similar arrests of growth (53). Primarily, these studies were made by radiographical examinations and case histories. However, experimental study of rats and dogs closely correlated with the radiographic information accumulated in clinical cases. Pollis (34) indicates growth arrest is common in animals under a wide variety of experimental nutritional diseases.

Because the bone and cartilage changes described by Cohen (16) and Harris (53) as arrest of growth produce a distinct histopathological entity this writer proposes the term "brachychondroplasia" (brachys = short + chondro = cartilage + plassein = to form) (111) be used to designate this condition. Brachychondroplasia is defined as a nonspecific disturbance of such severity that arrest of growth occurs. It is characterized by thin cartilage zones at the epiphysis, suppressed vascular invasion and cartilage destruction thin trabeculae in the spongiosa and suppression of osteoblastic function with deficient bone deposition. Mineralization is normal. Osteoid is present only as thin layers bordering the trabeculae and the cortex.

Microscopic examination of the costochondral junctions of the pigs from this experiment revealed lesions of both the disturbance in growth (eurychondroplasia) and arrest of growth (brachychondroplasia) described by Cohen (16).

The epiphyseal cartilage was much broader than normal in six pigs, numbers 3(30), 4(3), 2(7), 1(9), 4(9) and 5(9). This increase in breadth was principally in the vesicular cartilage zone. The chondrocytes continued to grow and multiply without vascular invasion and cartilage destruction. For normal mammalian bone growth, the broadening of the epiphyseal plate by growth and multiplication of the chondrocytes is balanced by a simultaneous vascular invasion by capillary

buds from the metaphysis. This process of uniform growth and destruction continues until maturity of the animal and closure of the epiphyseal line by ossification occurs.

Broad cartilage zones caused by suppressed vascular invasion are common in Iowa swine resulting hog cholera infections, hog cholera vaccinations and deficiencies in A vitamins according to Runnells et al. (127). Broad cartilage zones are also a characteristic lesion in classical and experimental rickets. Early investigators believed this cartilage zone was the result of excessive chondrocytic multiplication. However, Dodds and Cameroon (22) in careful studies showed the cartilage growth sequences were suppressed. Vascular invasion and destruction of the cartilage is believed suppressed because of a failure of provisional mineralization of the intercellular cartilage matrix and immaturity of the chondrocytes. Provisional mineralization of the epiphyseal cartilage was normal in the pigs examined during this experiment.

The epiphyseal cartilage was much narrower than normal in 8 pigs 4(30), 5(30), 6(30), 3(3), 4(7), 2(9), 3(9) and 6(9). This appearance is principally the result of narrow vesicular cartilage zones. Chondrocytic multiplication and growth is suppressed. Brachychondroplasia with arrest of growth has occurred.

Twelve of the fourteen pigs with abnormalities in the

vesicular cartilage zones, had areas of serous inflammation and necrotic cartilage foci in the vesicular cartilage zones and the primary spongiosa. Numerous mononuclear macrophages infiltrated these areas. Active congestion of the blood vessels was present. In focal areas the cartilage matrix became homogeneous and deeply eosinophilic in staining. The cartilage cells were necrotic in these areas.

In classical and experimental rickets, focal areas of necrotic cartilage occur as a result of compression and obstruction of the vascular system to these areas. Also much of the intercellular cartilage matrix becomes homogeneous, stains pale red with eosin and resembles very closely osteoid. The cartilage cells in the areas remain alive and in focal areas rejuvenation with multiplication occurs. Osteoblasts are numerous and active. Inflammation of the cartilage is not described in classical rickets.

All fourteen pigs with a marked microscopic osteodystrophy evidenced suppression of osteoblastic function, deficient deposition of bone, thin trabeculae and thin cortices. These changes are fundamentally the result of the disturbance of the osteoblasts. In most of these animals the osteoblasts were the inactive squamous or fibroblast types. The morphology of these cells suggest either a failure or incomplete differentiation of endothelial cells or dedifferentiation of func-

tional osteoblasts to a connective tissue cell (63). A similar condition is described in scurvy by Follis (36).

Suppression of osteoblastic function may result from a number of conditions and is therefore non-specific. It has been reported in inanition, copper deficiency, ascorbic acid deficiency, deficiencies of A vitamins and infectious diseases such as hog cholera.

Osteoid was either absent or present in thin layers along the trabecular borders of the metaphysis and on the periosteal border of the cortex. Two pigs 3(30) and 4(3) had focal areas of thick osteoid bordering the cortical bone of the diaphysis. These areas were not excessive and their position suggests they were remnants of prenatal or neonatal bone formation. Osteoid is normally abundant in the rapidly growing bones of the young infant (115) and could be expected in the newborn baby pig. There was no evidence of excess osteoid in the metaphysis, a characteristic lesion of classical rickets.

Extensive amounts of edematous reticular connective tissue were present in the intertrabecular spaces of the metaphysis of pigs 3(30), 4(3) and 1(3). Increased amounts of fibrous connective tissue is an irregular feature of classical rickets (Hess, 64). Marek and Wellmann (87) indicate in

juvenile rickets of animals greater amounts of fibrous connective tissue occurs than in classical rickets. They regard this increased amount of fibrous connective tissue as the result of greater mechanical stimulation to bone in animals.

Excessive amounts of fibrous connective tissues in the bone marrow is also termed fibrous osteodystrophy, osteofibrosis and osteitis fibrosa. Hutrya et al. (71) report osteofibrosis may occur secondary to rickets, osteomalacia or osteoporosis, resulting from mechanical stimulation to the weakened bone and possibly a special susceptibility of bone marrow or endocrine disturbance of individual animals and breeds. Lang (84) reports a similar fibrous osteodystrophy in rickets and osteoporosis in the human being.

In the human being, non-specific fibrous connective tissue hyperplasia in bone, Virchow's tissue (165) must be differentiated from three other specific fibrous osteodystrophic diseases (11), osteitis fibrosa (osteitis fibrosa cystica or von Recklinghausen's disease), osteitis deformans (Paget's disease) and fibrous dysplasia of bone. Osteitis fibrosa is the result of hyperparathyroidism and is usually associated with either an adenoma or hyperplasia of the parathyroid glands (11, 71). Osteitis fibrosa has been reported to occur in swine primarily affecting the cranial bones (71). Osteitis deformans and "fibrous dysplasia of bone" have not been re-

ported in animals.

Kowalczyk et al. (82) report a "fibrous dysplasia" of the cranial bones in pigs. The lesions were primarily limited to the nasal regions of these pigs. Extensive proliferation of the fibrous connective tissues was replacing the normal bone. Islands of bone tissues were surrounded by large irregularly outlined, multinucleate osteoclasts. Areas of hemorrhage were prominent and accumulations of hemosiderin were found scattered throughout the connective tissues. No descriptions of the ribs or long bones were presented. Kowalczyk suggested this condition was the result of a genetic factor. Trautmann as quoted by Hutyra et al. (71) found hyperplasia of the parathyroid glands in similarly affected pigs. Runnells et al. (127) indicate fibrous osteodystrophy is usually caused by renal disease, parathyroid hyperplasia or neoplasia and mineral deficiencies.

The amount of connective tissue in the bone marrow and metaphysis of the costochondral junctions from pigs in this experiment was moderate to slight. This connective tissue and its edema are believed associated with the degenerative changes in cartilage and possible mechanical stress.

Multinucleate syncytial cells were numerous in costochondral junctions of ten of the pigs examined. They were present bordering the trabeculae of the metaphysis, at the

zone of vascular invasion and cartilage destruction and the periosteal tissues of the cortex at epiphyseal-diaphyseal junction. These are the areas where cartilage destruction and bone formation is occurring, where mechanical stress is applied to the weak bone and the greatest reorganization of bone are occurring.

These multinucleate syncytial cells are also designated as osteoclasts and chondroclasts depending upon what tissues they associated. Their function is unknown, which has resulted in much controversy by histologists and pathologists. They resemble closely the foreign body giant cells which occur in chronic inflammation and are associated with the process of mineralized cartilage and bone destruction. The point of greatest controversy is whether they are active in bone breakdown or whether they are passively stimulated by bone and cartilage breakdown. Copenhaver and Johnson (17) indicate there is no direct evidence that the multinucleate syncytial cells are active agents in this process and absorption of bone may take place in the absence of these cells. Ham (51) suggests that the multinucleate syncytial cells result from the fusion of degenerating osteoblasts which have become nonfunctional. He believes functional osteoblasts and osteocytes are necessary for maintenance of bone. If this function is disturbed, tissue fluids begin osteolytic processes

to remove to bone. Weinmann and Sicher (163) suggest the osteoclasts secrete proteolytic enzymes which destroy the bone matrix allowing the bone salts to be removed by tissue fluids or phagocytized by mononuclear macrophages. Hancox (52) states that the balance of evidence indicates osteoclasts cause absorption of bone by superficial or lacunar erosion. His report has a very extensive discussion on osteoclasts. Follis et al. (37) indicate numerous osteoclasts are common in costochondral junctions of normal pigs and are an indication of rapid growth processes. Osteoclasts when present in large numbers are usually associated with increased absorption of bone. This is most frequently observed in the fibrous osteodystrophic diseases of man and animals. Osteoclasts are also numerous in the tissues of organizing bone fractures.

Bone marrow activity was suppressed in all fourteen pigs evidencing osteodystrophy of the costochondral junctions. This indicates hematopoietic function in these animals was impaired. Because this suppression is directly correlated with the disturbance in bone and cartilage growth of these pigs, it is believed they are the result of the same etiologic agent.

Microscopic examination of four pigs, 1(3), 2(3), 1(7), and 3(7) revealed normal well-ossified bone. The cartilage zones were normal in breadth. Active growth and multiplication of the chondrocytes were present. The pattern of vascu-

lar invasion and cartilage destruction was regular. The capillary buds were actively growing and very cellular. Osteoblasts were numerous and the active cuboidal type. Osteoid deposited on the borders of the trabecuale was quickly ossified. The cortex was thick. The periosteum was thin and contained few osteoblasts and fibrocytes. The appearance of the bone in these animals indicates the process of normal growth has resumed. The earlier inhibiting effect of the unknown factor causing the edema has been effectively overcome by these animals. It is believed that these animals would have been able to develop lesions of rickets if it had been going to occur. However, because this experiment was complicated by generalized edema, retarded growth and deaths, this experiment should be repeated in its original form and with modifications to accurately determine the effect of deficiency of D vitamins in baby pigs and evaluate the role of this ration in the clinical edema syndrome observed.

Microscopic examination of the distal ends of the femur and promixal ends of the tibia in the rats fed the U.S.P. Rachitogenic Diet no. 2 revealed the characteristic lesions described in advanced experimental classical rickets. The vesicular cartilage zone was broad with long "tongues" projecting into the metaphysis. The cartilage columns were bent and the chondrocytes compressed. Thick osteoid trabecu-

lae containing central cartilage cell cores formed the metaphysis. Osteoblasts were numerous along the vascular channels nearest the epiphyseal plate. These blood vessels in the metaphysis had a definite "bush" arrangement. The haversian canals of the cortex were almost occluded by thick osteoid layers. The periosteum was thick.

The production of experimental rickets in these rats proved the environment, light source and control rats used in this experiment were satisfactory for the production of classical rickets, the purpose of this experiment.

Rats fed the synthetic milk diet, Ration A, grew rapidly. Microscopic examination of bones from these rats revealed normal well-ossified bone. Excessive amounts of cartilage and osteoid were absent. Formation and destruction of cartilage and formation of bone were proceeding in a normal orderly fashion. Osteoblasts were numerous and active. Vascular invasion was occurring normally.

These results indicate the experimental ration was complete in all respects except D vitamins. Because Ration A contained ample amounts of calcium and phosphorus in a ratio of approximately 1:1, these rats were able to utilize the minerals present on a D-vitamin deficient ration. These results support the observations reported by Nicolaysen and Eeg-Larsen (108) that young rats are able to assimilate cal-

cium and phosphorus without D vitamins in the ration and witnout exposure to ultraviolet light rays if these minerals are provided in a favorable ratio and in a readily available form.

Rats fed the control A vitamin deficient test diet developed characteristic lesions of severe A vitamin deficiency (65, 149). Microscopic examination revealed brachychondroplasia. The cartilage zones were thin. Mineralization was normal. Bone deposition had ceased. The trabeculae and cortices were thin. Numerous mast cells were observed in the intertrabecular spaces. Follis (34) has shown these mast cells are a non-specific manifestation peculiar to the rat and seen in a variety of experimental conditions. He was unable to determine a reason for their appearance.

Chicks fed the U.S.P. Rachitogenic Diet No. 2 were retarded in growtn and feathered poorly. Microscopical examination of bones from these chicks revealed a broad epiphyseal cartilage with thick layers of osteoid in the metaphysis and cortex. These lesions indicate the presence of avian rickets in these birds. Avian rickets resemble in many respects that of experimental classical rickets in rats. However, anatomical and physiological differences in the growth of bone in the chick prevent the characteristic cellular pattern observed in classical rickets. The presence of avian rickets in these

birds confirm the results of the rats on this same diet.

The chicks fed the synthetic milk diet, Ration A, grew rapidly and feathered well. Microscopical examination of the bones from these birds revealed excessive amounts of osteoid principally along the periosteal surface of the cortex. The epiphyseal cartilage was thin. Vascular invasion was extensive and numerous wide vascular channels were present. Osteoblasts were numerous and the active cuboidal form. The cortex was thin. The mechanical stress at the ephiphyseal-diaphyseal junction resulted in microfractures with numerous multinucleate syncytial cells and a periostitis with connective tissue proliferation. The results from these chicks suggest a mild form of rickets was present. Because the ration fed was complete in all respects except D vitamins and contained calcium and phosphorus in a 1:1 ratio, the effext of the D vitamin deficiency was modified. The results from these birds indicate the ration fed to the pigs was deficient in D vitamins and complete in all other respects. Therefore it is believed, the ration was satisfactory for the study of D vitamin deficiency in baby pigs.

SUMMARY AND CONCLUSIONS

1. Twenty baby pigs were fed synthetic milk rations deficient in D vitamins in the absence of sunlight and artificial ultraviolet light rays.

2. Clinical observation revealed severe retardation in growth, unthriftiness, rough hair coats and extensive subcutaneous edema. This edema was pronounced in the ventral subcutaneous tissues of the thighs, hocks and submandibular and ventral abdominal regions. In severe cases, the skin was thrown into distinct folds and had dried serum exudate on the surface. The ears were thick and rolled. Excitability or listlessness and mild incoordination was observed followed by depression and generalized weakness of increasing severity. When this weakness became severe enough that the pig was unable to eat or stand, death followed in 6 to 12 hours. Three pigs survived the edematous stage of this experiment.

3. Four pigs exhibited a clinical osteodystrophy. This osteodystrophy was primarily manifest by the hocks or the digits of the front and hind feet being turned medially. The most severely affected pig was the most rapid growing and largest individual.

4. Three pigs had broad white lines at the costochondral junctions upon post mortem examination. Microscopic examination revealed these areas consisted of broad vesicular and proliferating cartilage zones with irregular vascular in-

vasion and cartilage destruction.

5. Microscopic examination revealed variable disturbances in bone and cartilage growth in fourteen of eighteen pigs examined. The remaining four pigs evidenced normal bone and cartilage growth. The principal lesions observed were variation in the breadth of the epiphyseal cartilage plate, irregular vascular invasion and cartilage destruction, thin trabeculae, thin cortices and suppression of osteoblastic function with a resulting deficient bone deposition. No excess osteoid was present.

6. Comparison of the clinical, gross and microscopic appearance of these pigs to classical rickets revealed no evidence of this disease.

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The author wishes also to express his appreciation to Dr. P. C. Bennett, Supervisor of the Iowa Veterinary Medical Diagnostic Laboratory, and Dr. F. K. Ramsey, Head of the Department of Veterinary Pathology, for their cooperation during this study.

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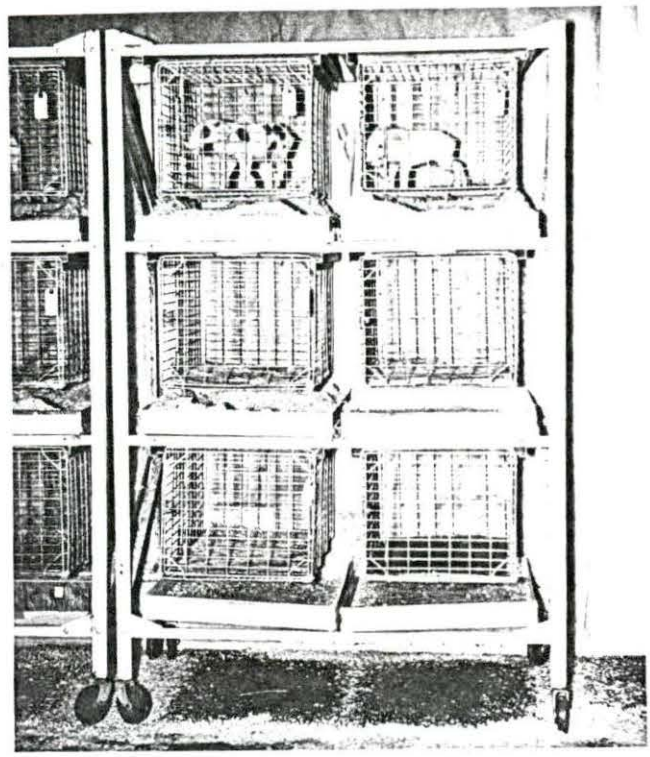
The author gives special credit to his wife, Judy, for her encouragement and helpful assistance during the course of this study and the preparation of this thesis.

APPENDIX

Figure 1. A six rabbit cage unit with masonite floors used for pens during tnis experiment.

Figure 2. The thermostatically controlled glassware oven used for heat.



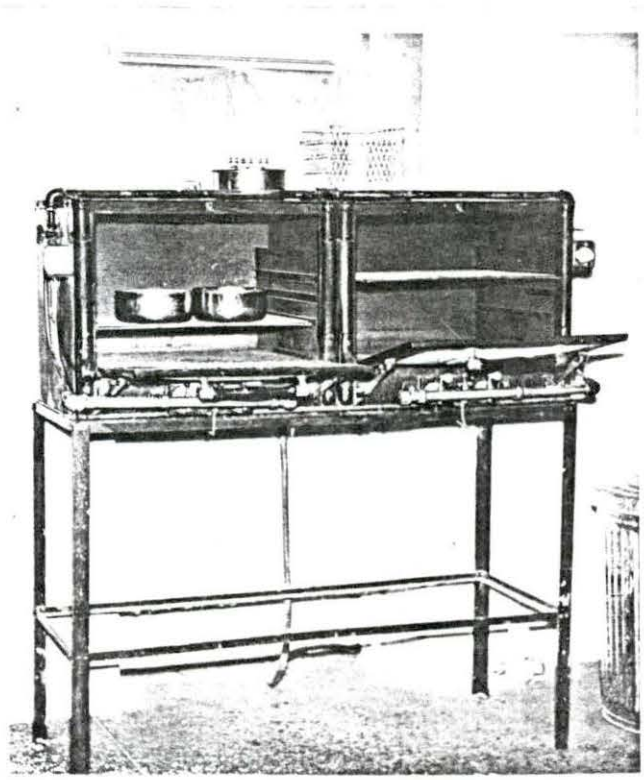
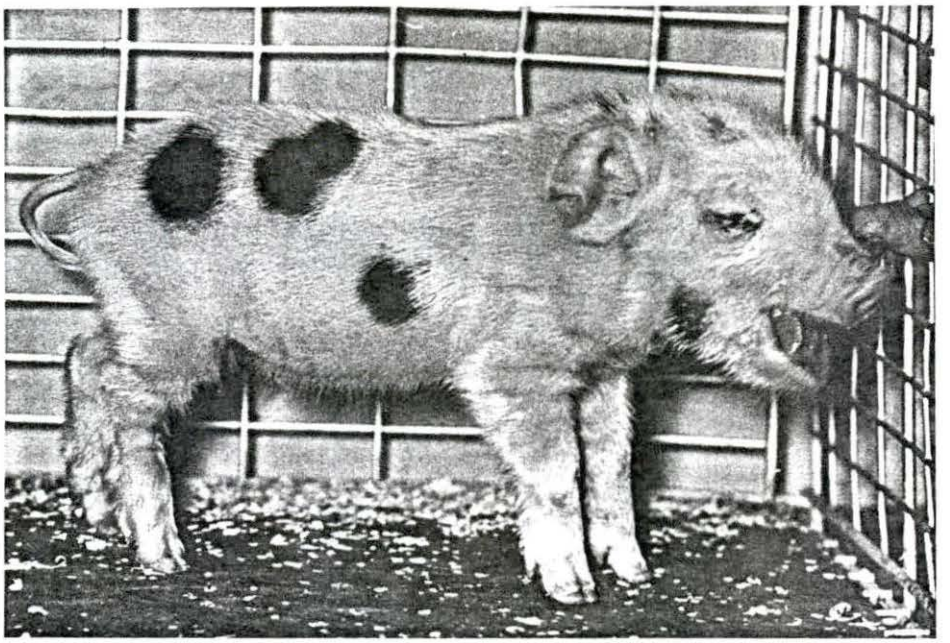


Figure 3. Pig 1(3), age, 20 days: Note the rough hair coat, rolled ears and folds in the skin.

Figure 4. Pig 2(3), age, 20 days: Note the unthrifty appearance of this pig.



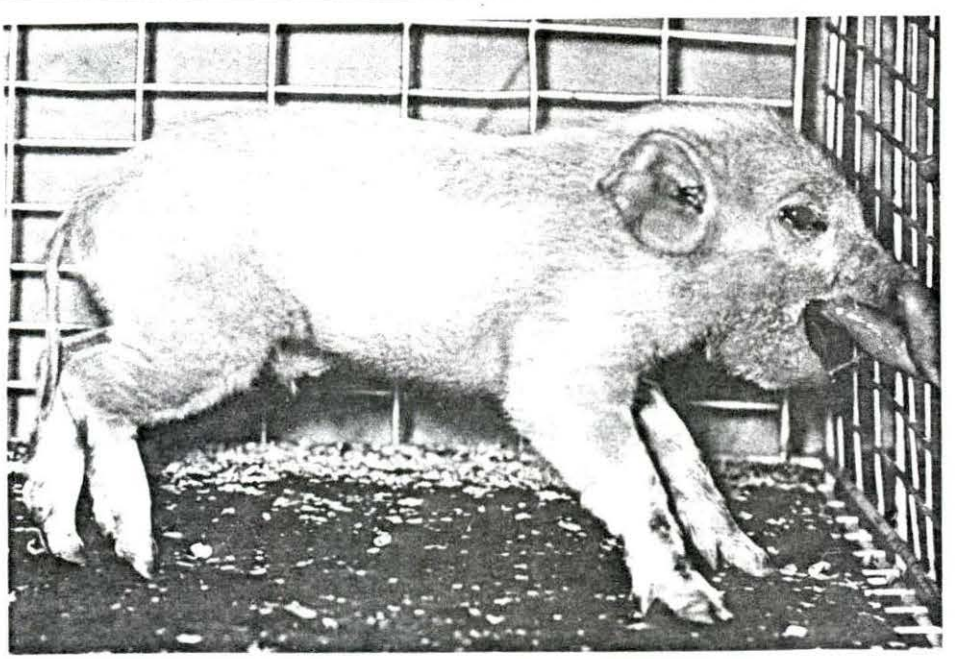
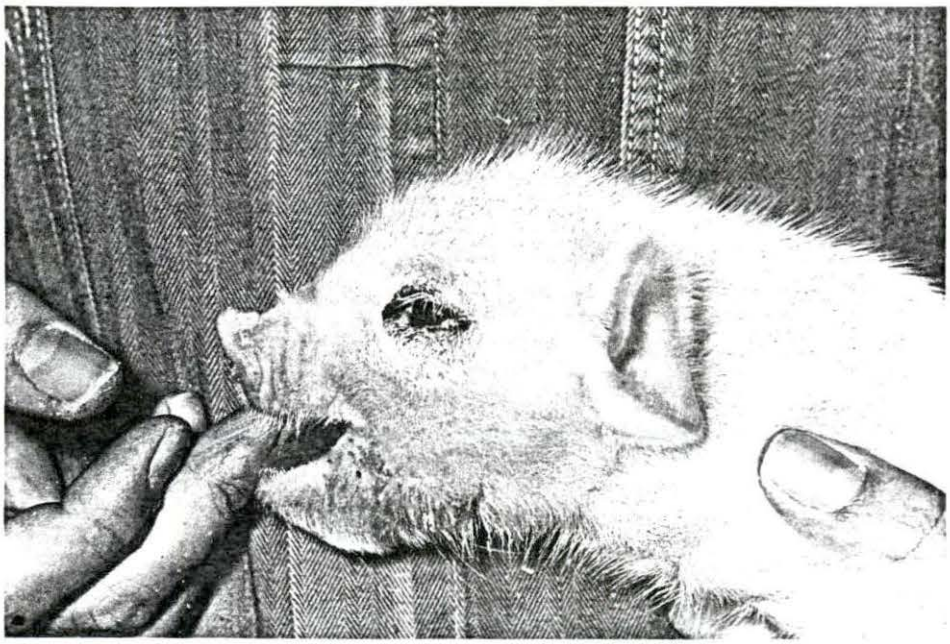


Figure 5. Pig 1(9), age, 16 days: Note the pendulous folds of the skin in the submandibular and ventral cervical regions.

Figure 6. Pig 3(7), age, 20 days: Note the folds in the skin at the hock and the position and shape of the front legs.



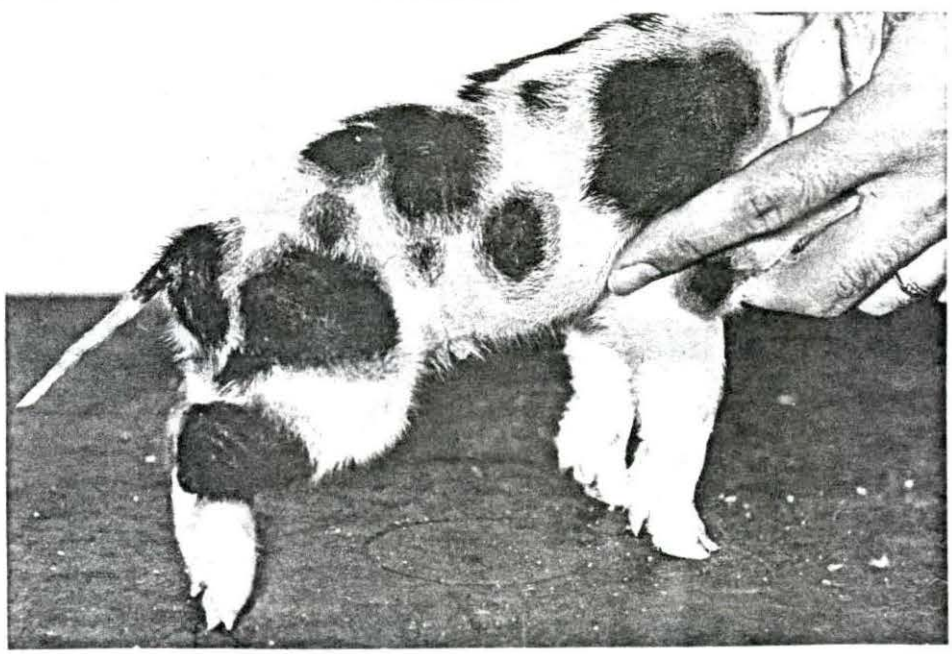


Figure 7. Pig 1(3), age, 22 days: Severe subcutaneous edema of the stifle.

Figure 8. Pig 1(3), age, 26 days: Slight subcutaneous edema of the stifle. Note the dry serum exudate on the surface of the skin.

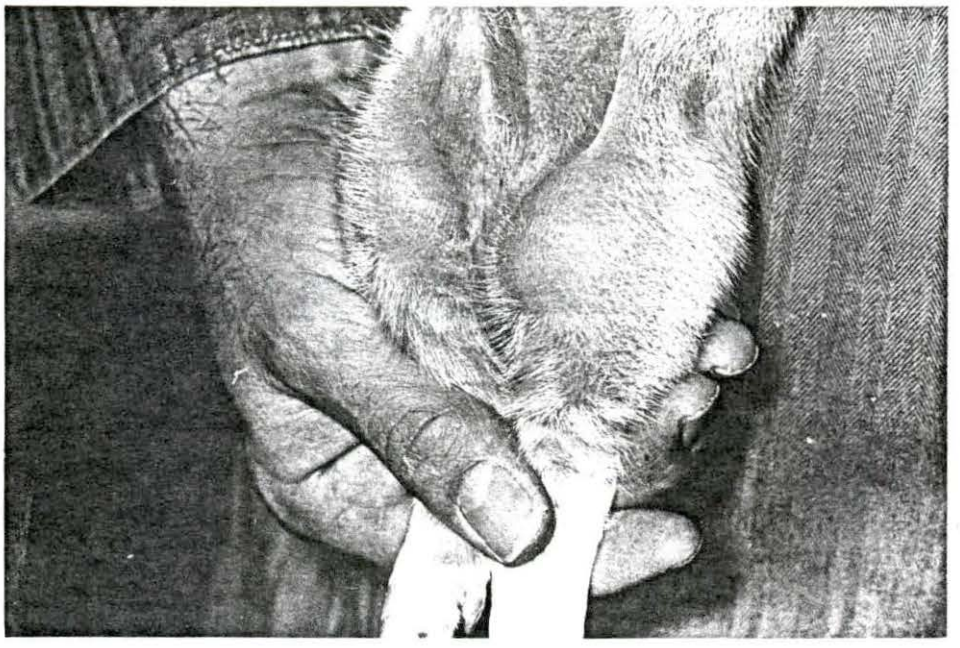
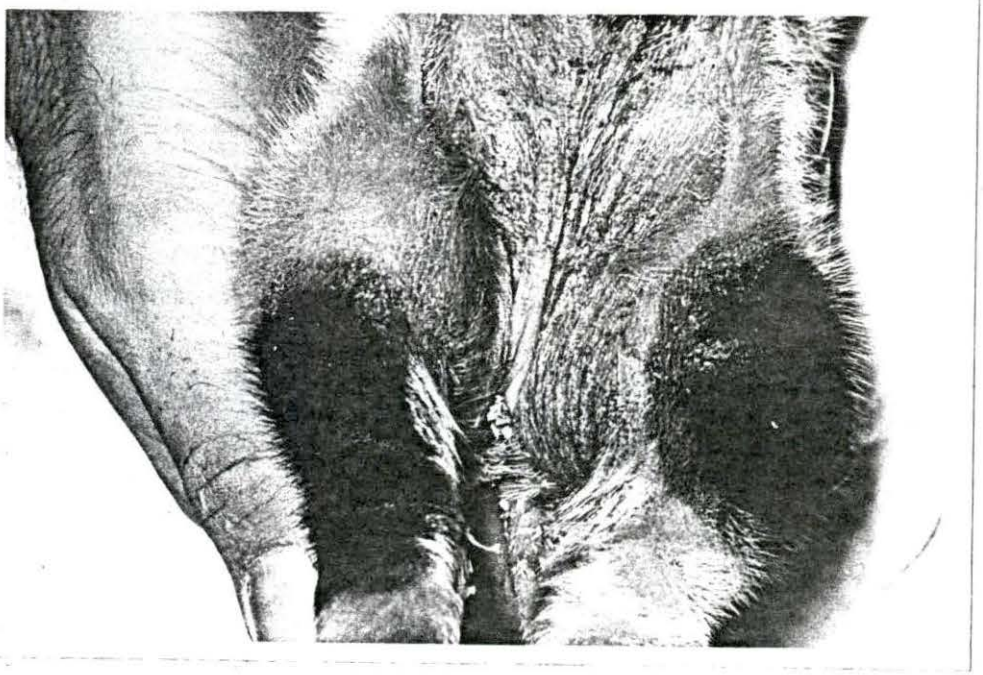


Figure 9. Pig 3(7), age, 16 days: Severe subcutaneous edema of the stifles and hocks. Note the prominent folds in the skin at the hock.

Figure 10. Pig 3(7), age, 22 days: Slight subcutaneous edema of the stifles.



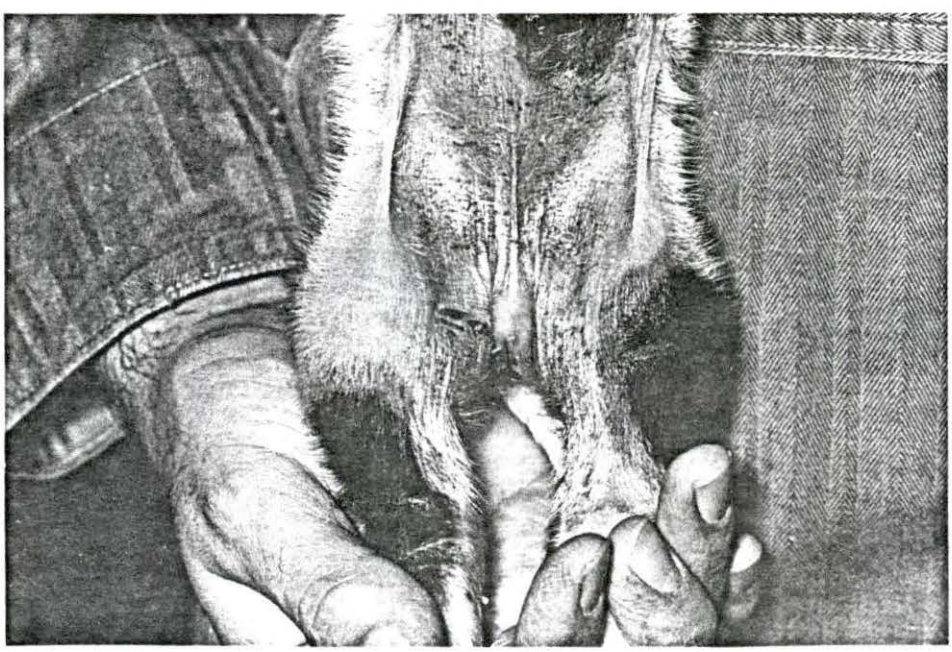


Figure 11. Pig 1(9), age, 14 days: Note the edema of the umbilicus and the extensive dry serum exudate on the surface of the skin.

Figure 12. Pig 2(3), age, 56 days: Note the size of this pig in relation to the amount of cage space. Compare this figure with Figure 4.

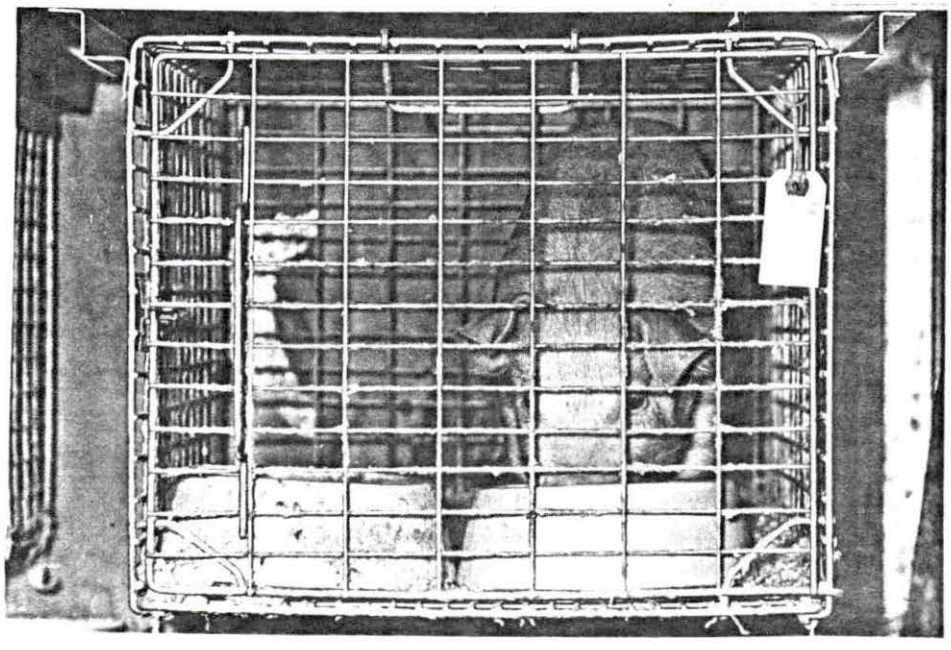
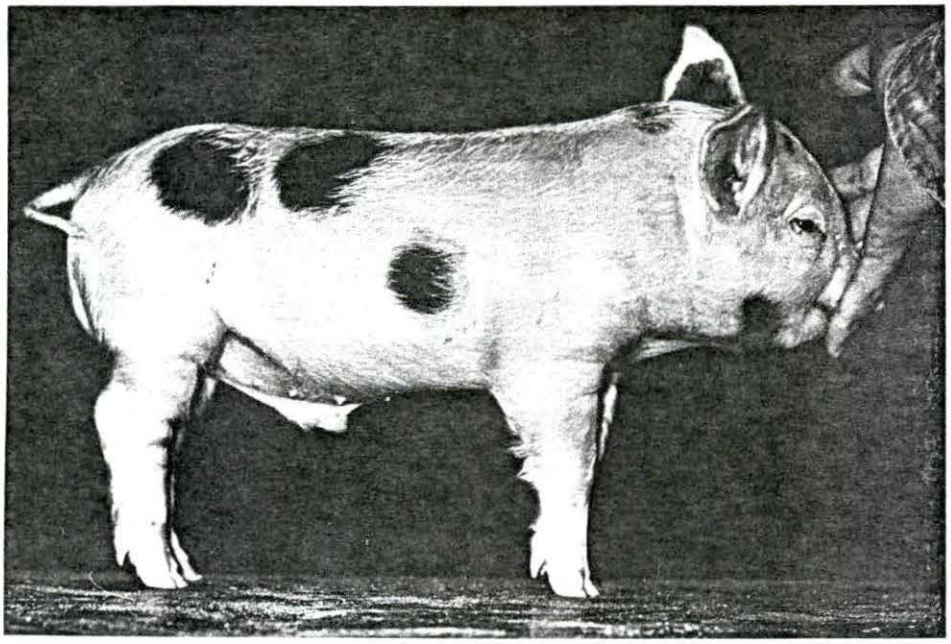


Figure 13. Pig 1(3), age, 56 days: Note the thrifty appearance of this pig. Compare this figure with Figure 3.

Figure 14. Pig 2(3), age, 56 days: Note the thrifty appearance of this pig. Compare this figure with Figure 4.



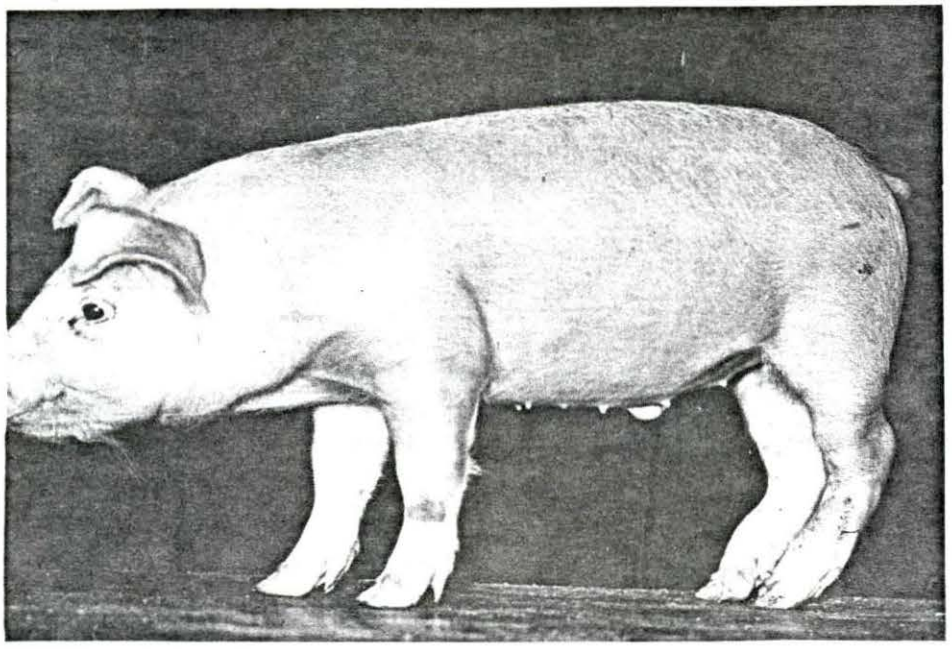
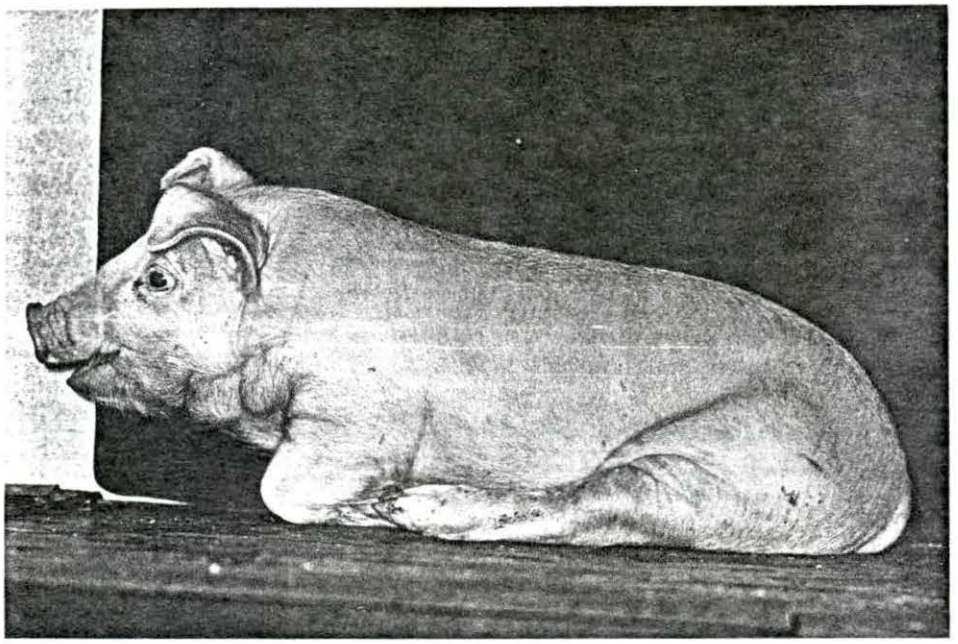


Figure 15. Pig 2(3), age, 56 days: The resting position of this pig with the hind legs extended anteriorly along side of the body.

Figure 16. Pig 2(3), age, 56 days: Note the bowed appearance of the front legs and the irregular curvature of the hind legs.



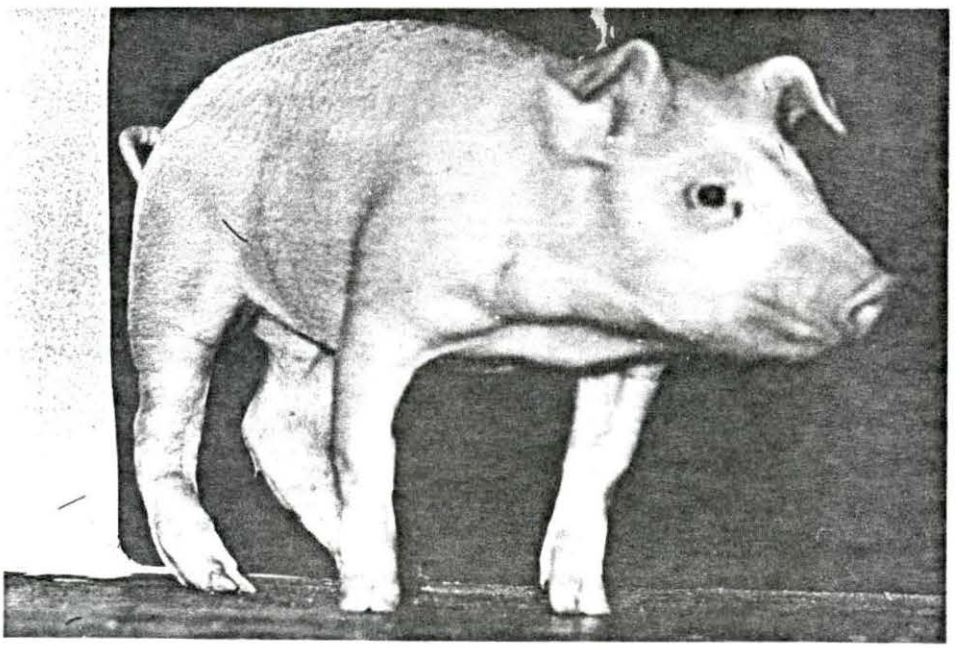
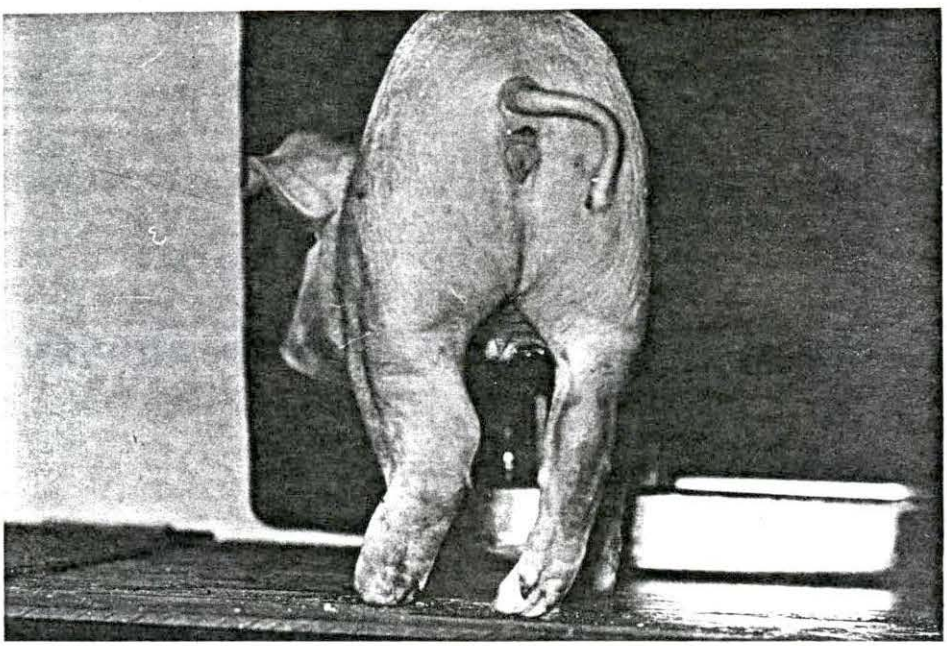


Figure 17. Pig 2(3), age, 56 days: Posterior view of the hind legs. Note the shape and position of the hocks and digits.

Figure 18. Pig 2(3), age, 56 days: Lateral view of the hind legs. Note the shape and position of the stifle, hock and digits.



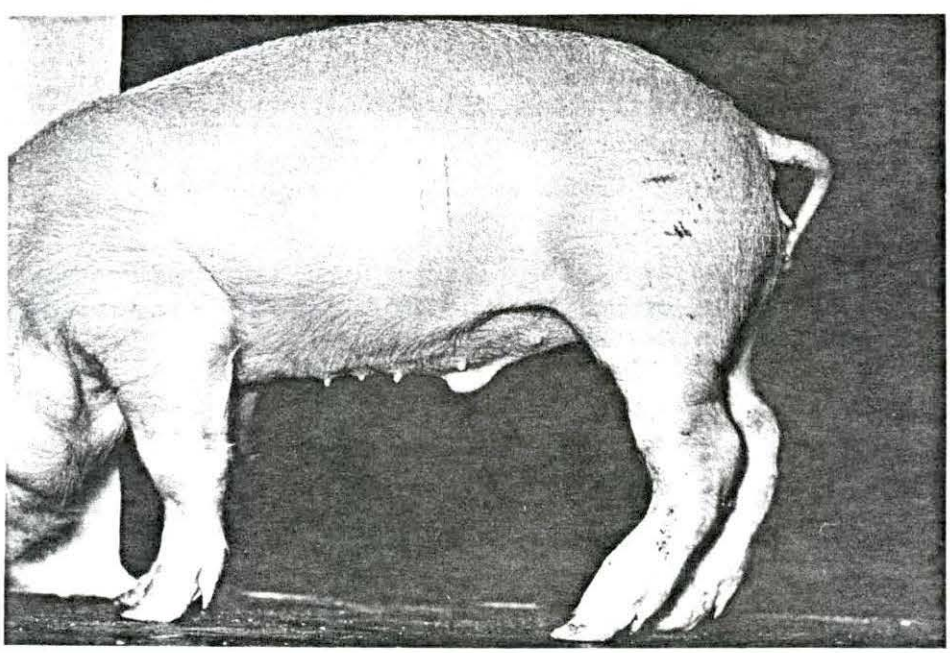
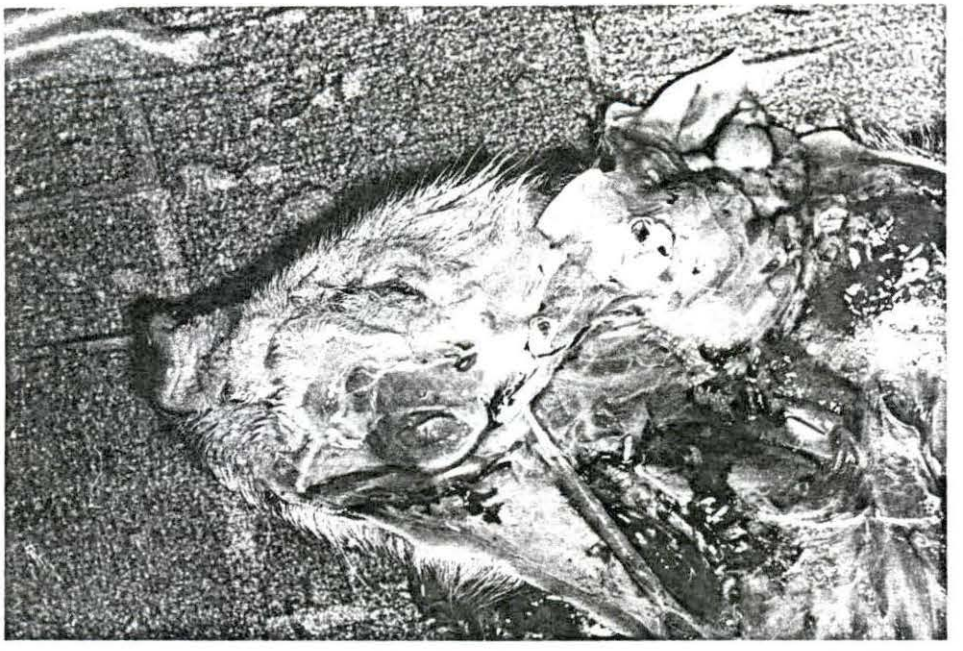


Figure 19. Pig 3(9), age, 14 days: Note the wet appearance of the tissues.

Figure 20. Pig 3(9), age, 14 days: Note the extensive subcutaneous edema of the connective tissues of the ventral abdominal wall.



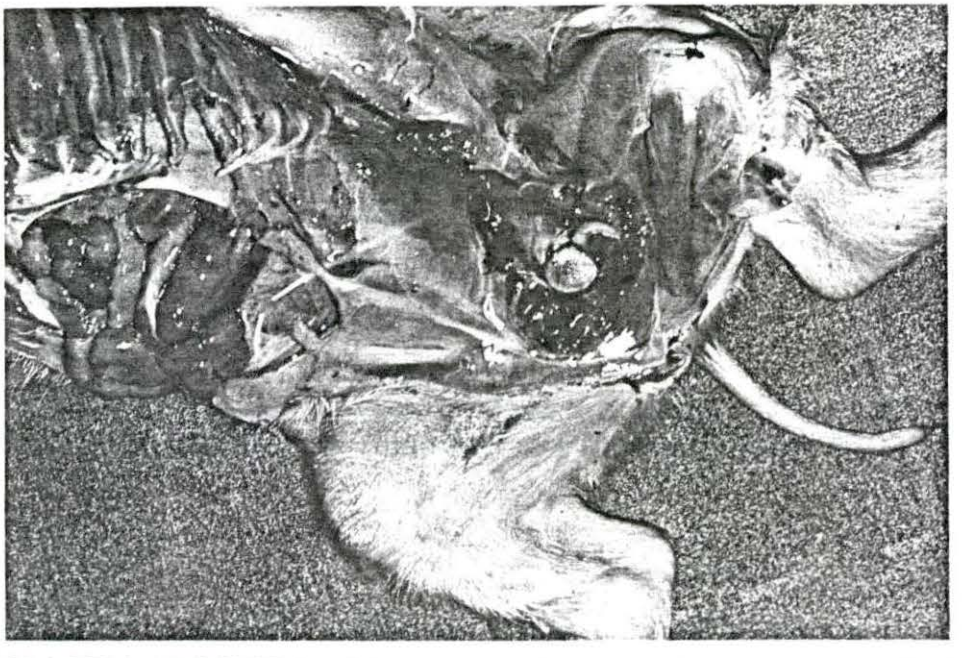


Figure 21. Pig 3(9), age, 14 days: Severe edema of the subcutaneous connective tissues of the back.

Figure 22. Pig 3(9), age, 14 days: Severe edema of the subcutaneous connective tissues of the neck.

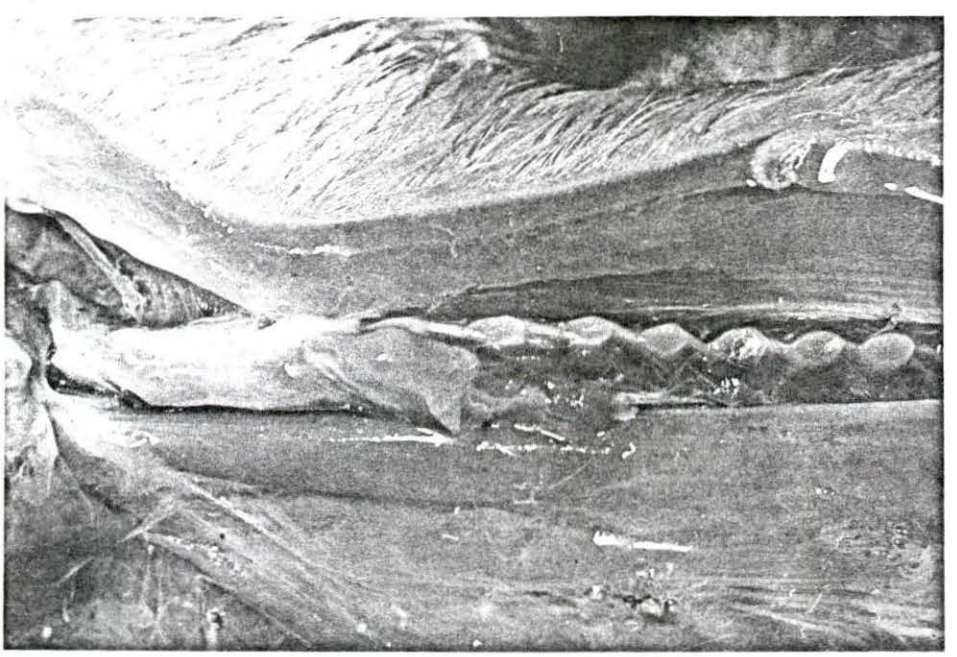
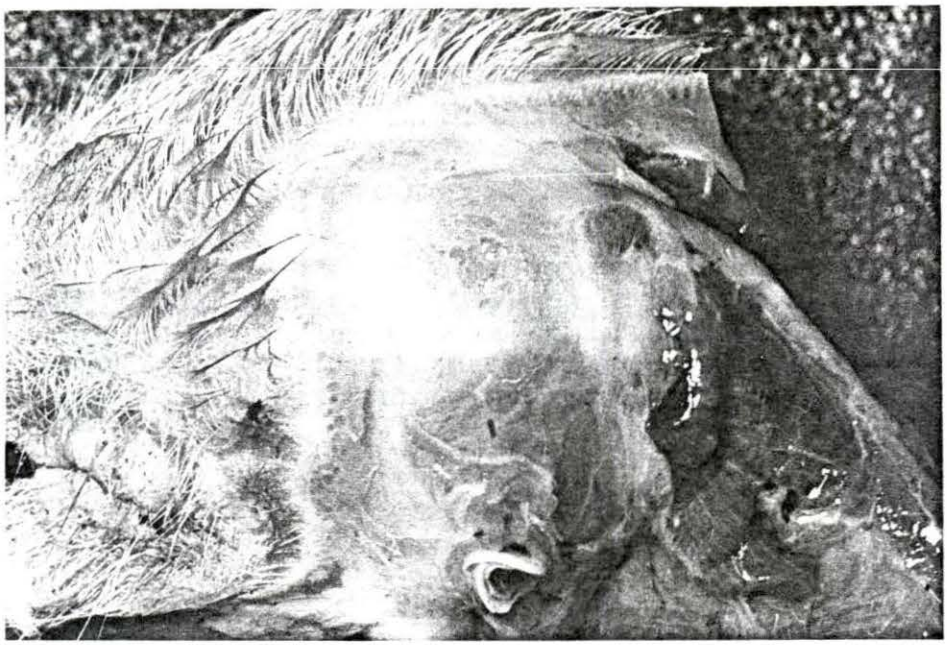


Figure 23. Pig 3(9), age, 14 days: Note the thickness of the edematous subcutaneous connective tissues of the skin at the back of the head.

Figure 24. Pig 2(7), age, 6 days: Note the prominent broad white line at the costochondral junctions.



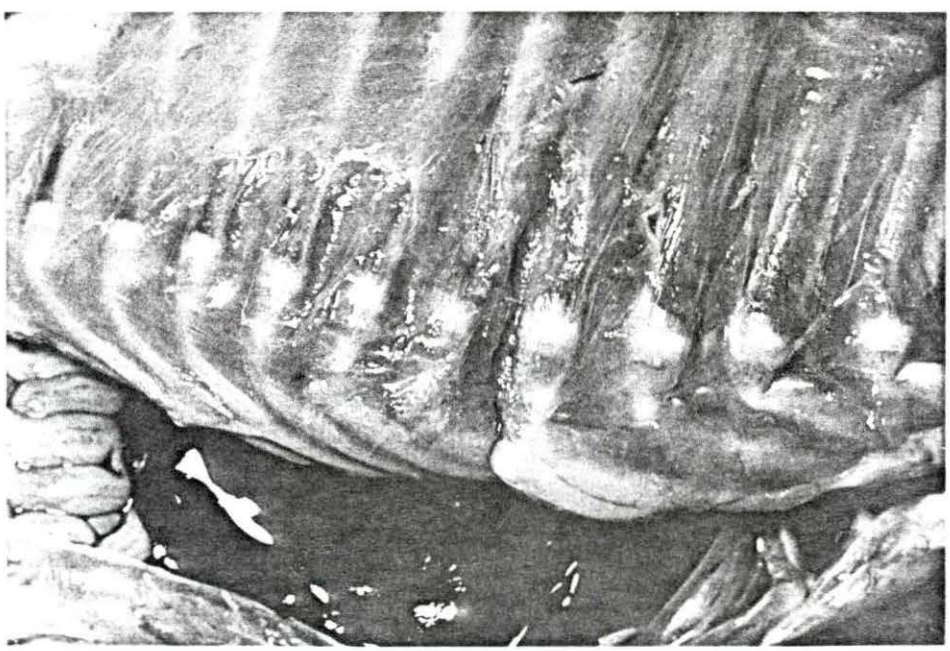
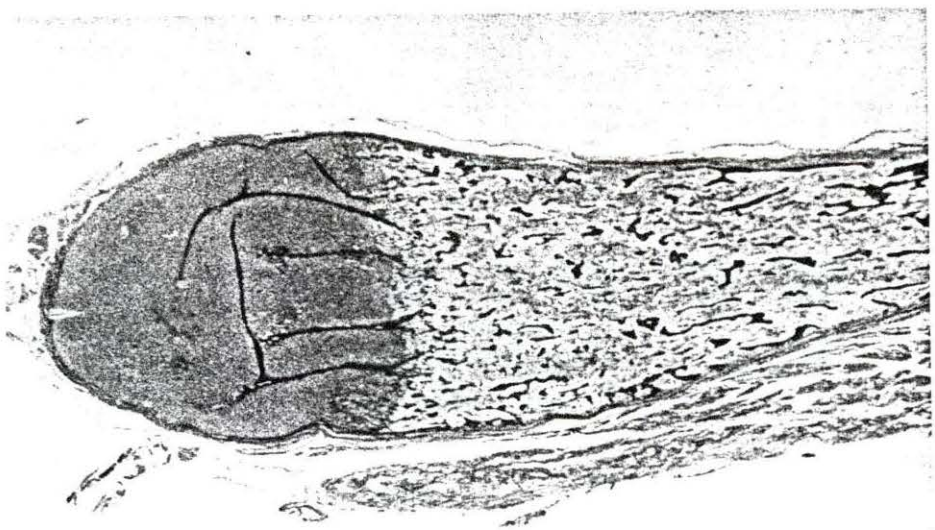


Figure 25. Pig 6(30), age, 16 days: The epiphyseal plate of cartilage is normal in appearance. The pattern of vascular invasion and cartilage destruction is regular. Note the thin appearance of the cortex. x 13.

Figure 26. Pig 3(30), age, 4 days: The epiphyseal plate of cartilage is very broad. The pattern of vascular invasion and cartilage destruction is very irregular. x 13.



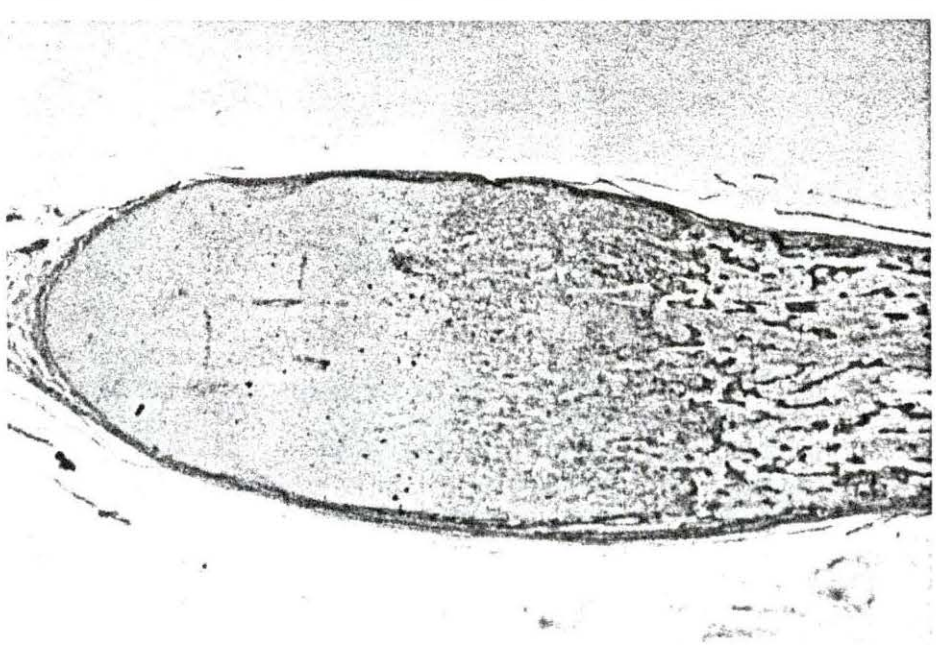
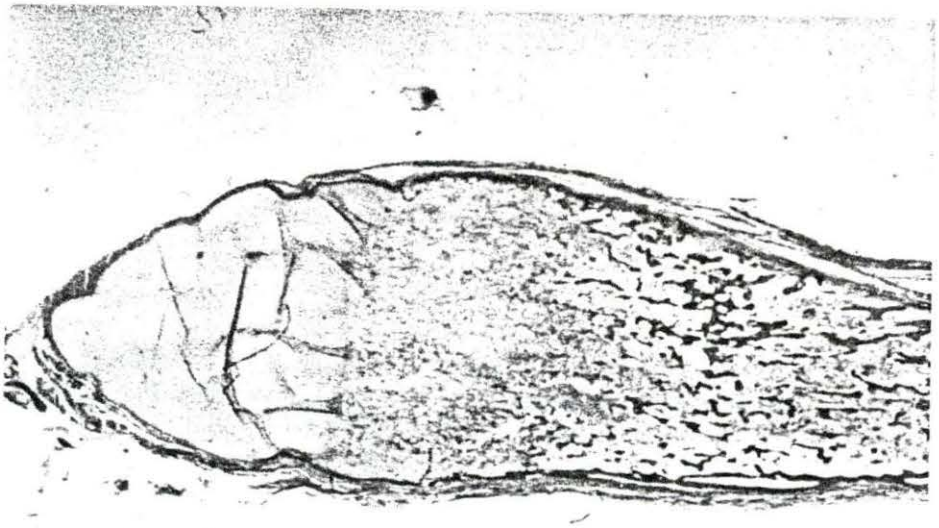


Figure 27. Pig 4(3), age, 10 days: The epiphyseal plate of cartilage is very broad. The pattern of vascular invasion is very irregular. Note the sharp line of demarcation between the proliferating zone of cartilage and the vesicular zone of cartilage. x 13.

Figure 28. Pig 4(30), age 4 days: The epiphyseal plate of cartilage is normal in appearance. The pattern of vascular invasion and cartilage destruction is regular. Compare the thick trabeculae and cortex of this animal with those of Pig 6(30), Figure 25. x 13.



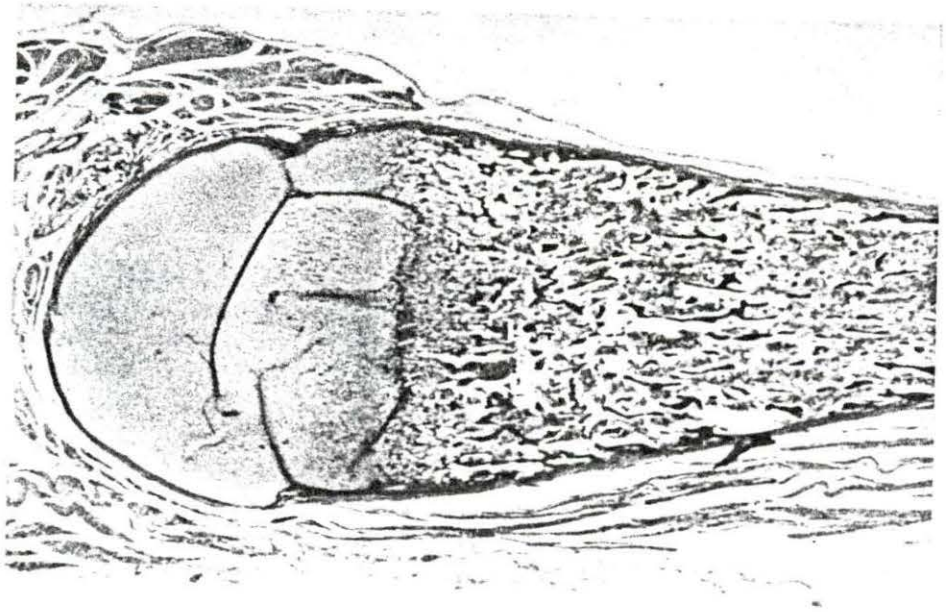
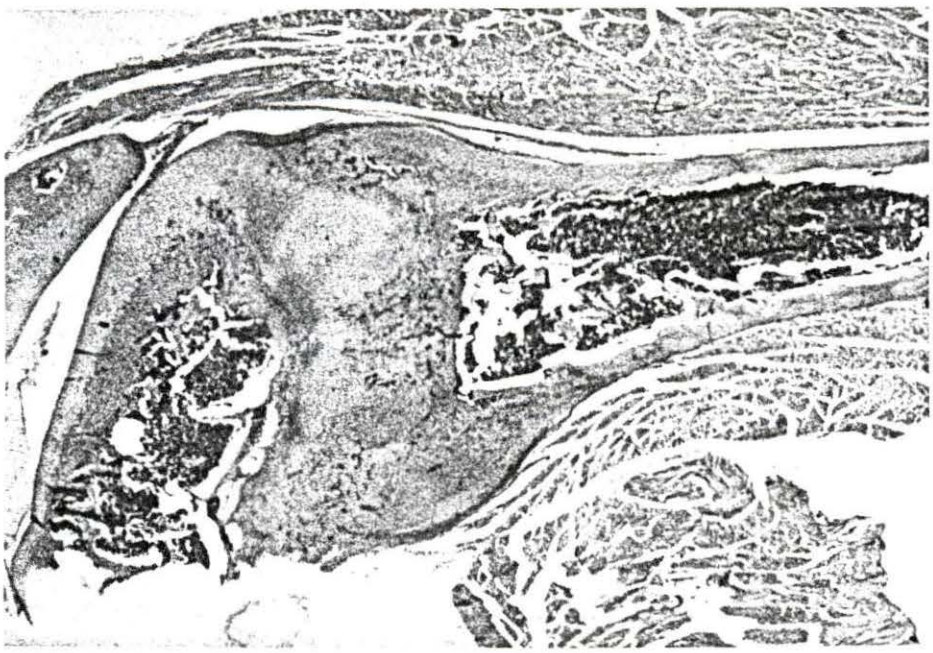


Figure 29. Rat (R-71), age, 93 days: The tibia of a control rat with severe experimental classical rickets. Note the broad epiphyseal plate of cartilage, the broad metaphysis and the thick cortex. Compare this figure with the tibia of Rat (A-47), Figure 30. x 11.

Figure 30. Rat (A-47), age, 69 days: The tibia and femur of a control rat with brachychondroplasia resulting from a severe deficiency in A vitamins. Note the thin epiphyseal plate of cartilage and the thin cortex. Compare this figure with Rat (R-71), Figure 29. x 11.



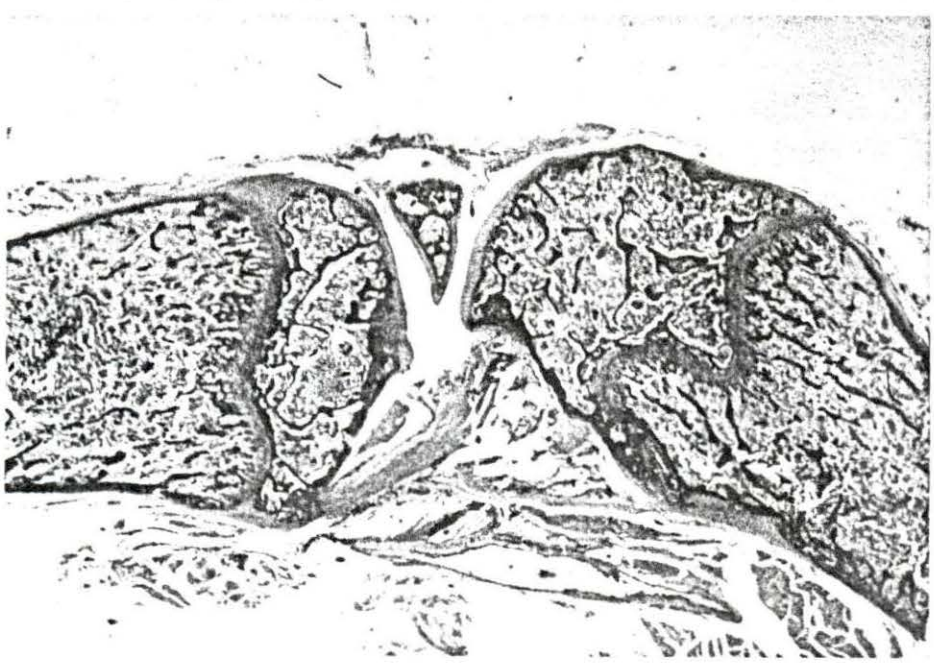
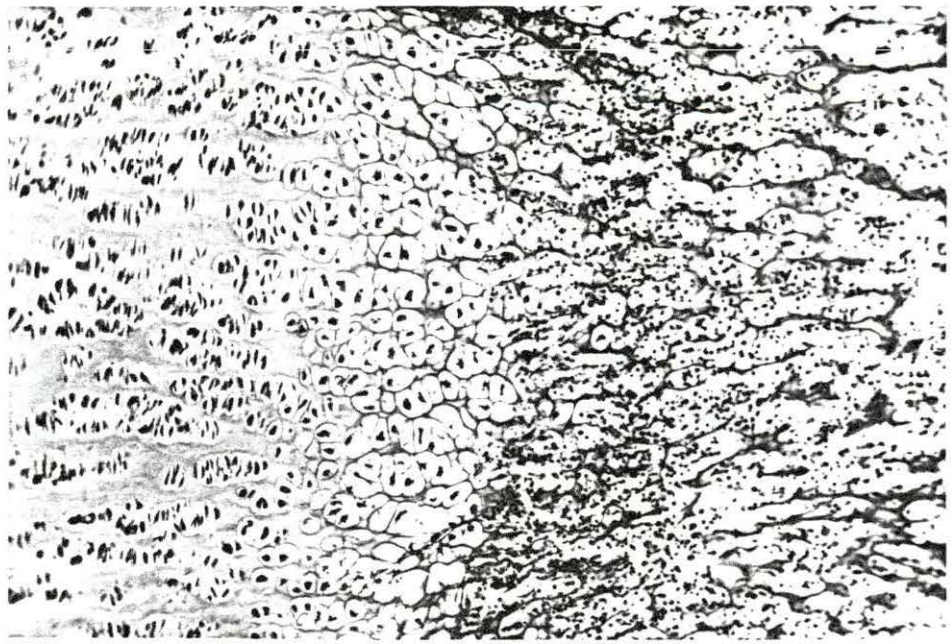


Figure 31. Pig 4(30), age, 4 days: The vesicular zone of cartilage is thin. Vascular invasion is occurring in a regular pattern. The metaphysis is composed of a lattice of thin osteochondral trabaculae with deficient deposition of bone. x 166.

Figure 32. Pig 4(3), age, 10 days: The vesicular zone of cartilage is very broad with an irregular pattern of vascular invasion and cartilage destruction. Note the sharp line of demarcation between the vesicular zone of cartilage and the proliferating zone of cartilage. x 66.



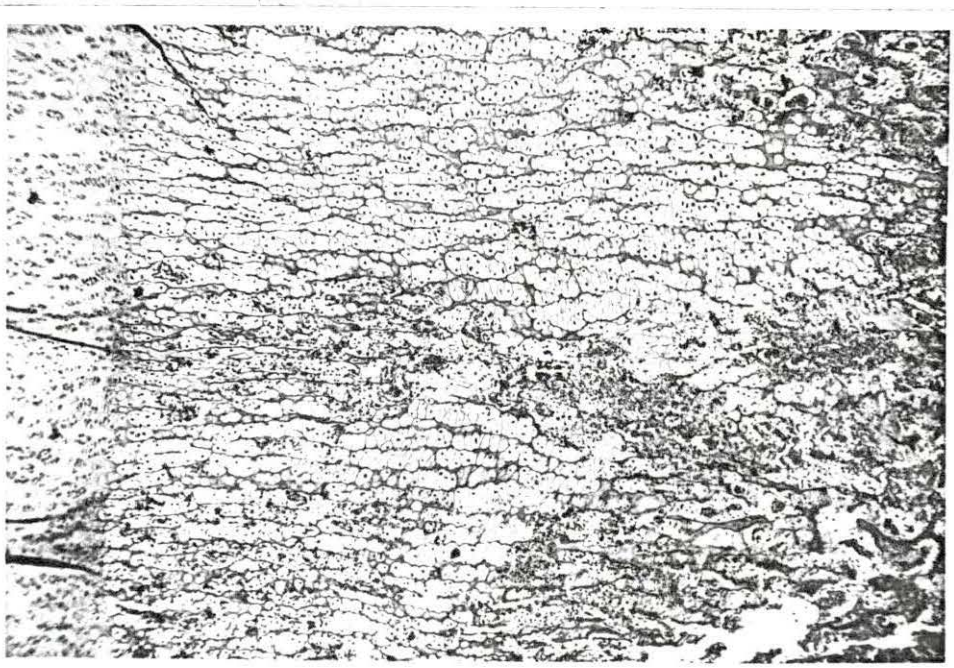
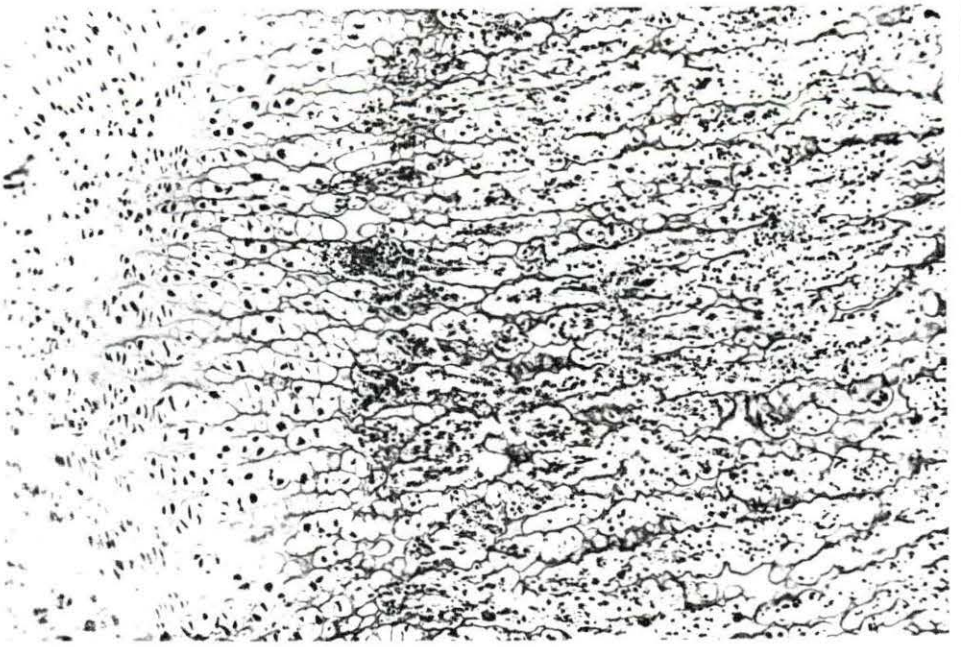


Figure 33. Pig 5(30), age, 11 days: The vesicular zone of cartilage is narrow. The pattern of vascular invasion and cartilage destruction is regular. Note the absence of osteoblasts, the deficient deposition of bone and the lattice of thin trabeculae which form the metaphysis. x 166.

Figure 34. Pig 3(7), age, 42 days: Normal epiphyseal cartilage. Note the straight columns of chondrocytes in vesicular zone of cartilage. Compare this figure with Pig 5(9), Figure 33. x 166.



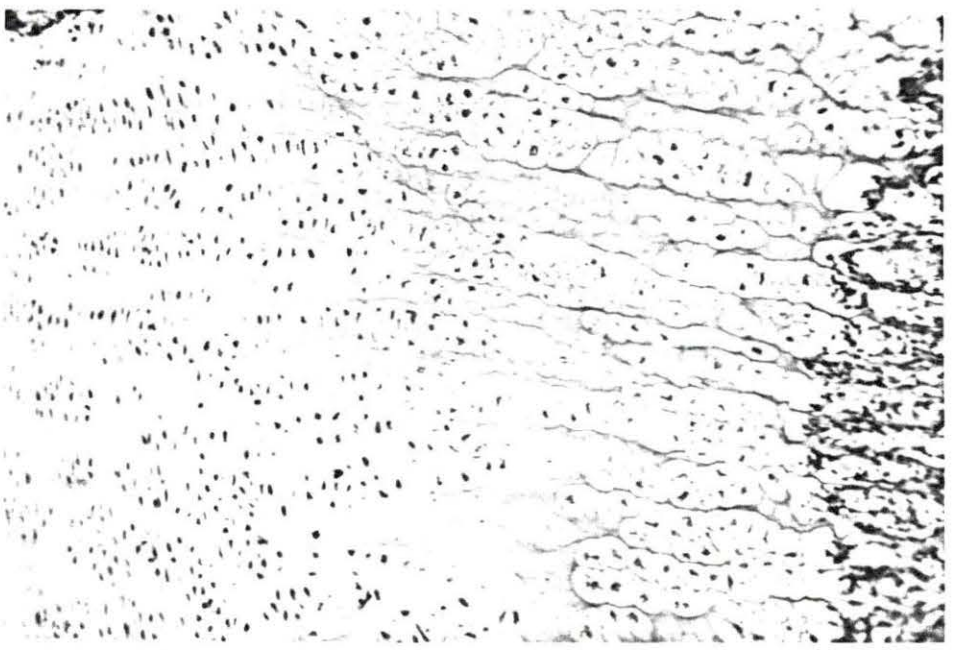
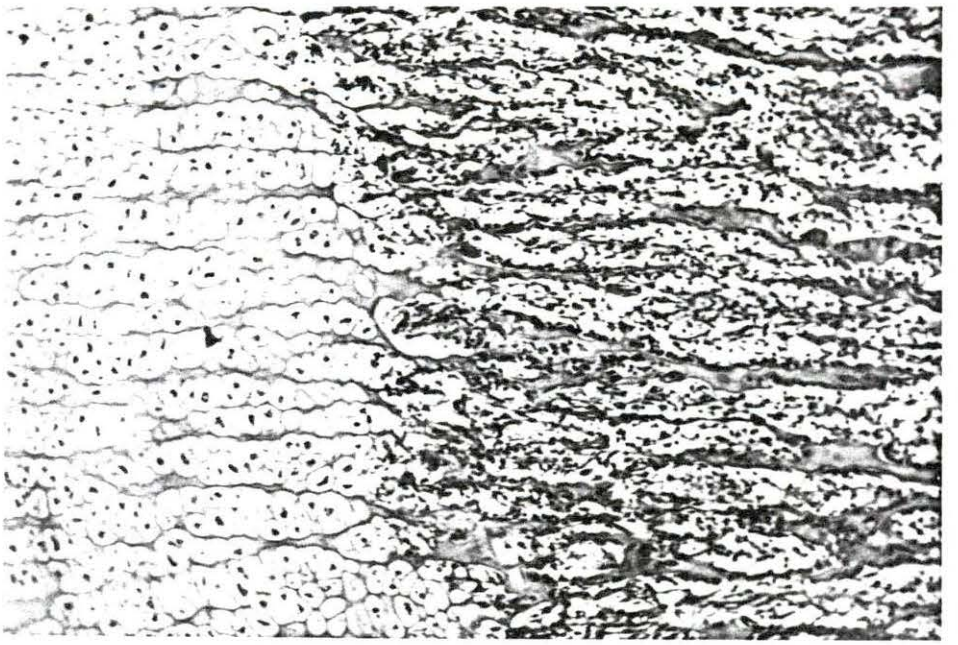


Figure 35. Pig 3(7), age, 42 days: A normal metaphysis with a regular pattern of vascular invasion and cartilage destruction. Note the density of the metaphysis caused by the presence of numerous active osteoblasts and actively congested blood vessels. Compare this figure with Pig 5(30), Figure 33 and Pig 4(30), Figure 31. x 166.

Figure 36. Pig 5(9), age, 12 days: Note the narrow vesicular zone of cartilage, the absence of osteoblasts and the irregularly shaped and sized osteochondral trabeculae. Compare this figure with Pig 3(7), Figure 35. x 166.



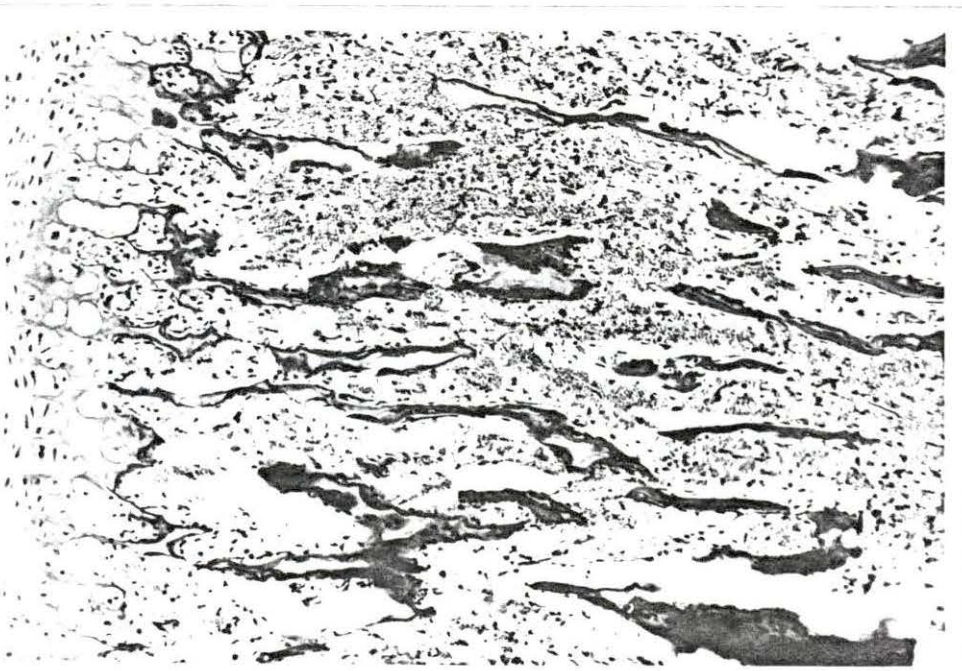
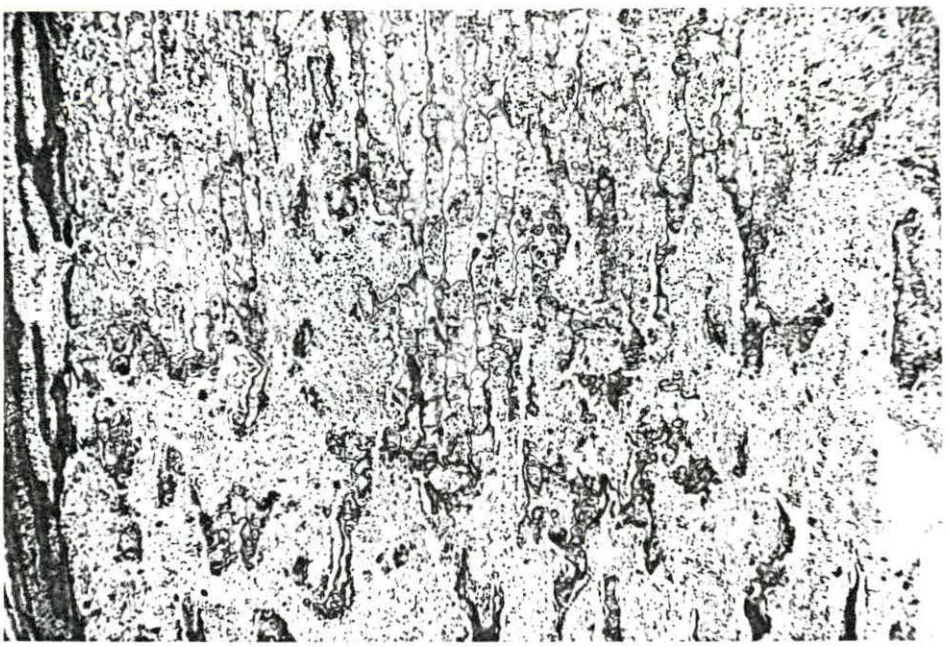


Figure 37. Pig 3(30), age, 4 days: An irregular pattern of vascular invasion and cartilage destruction. Note the irregularly shaped osteochondral trabeculae containing necrotic and mineralized cartilage. x 66.

Figure 38. Pig 3(9), age, 14 days: A regular pattern of vascular invasion and cartilage destruction. Note the absence of osteoblasts and capillary buds, the deficient deposition of bone, the thin osteochondral trabeculae and the severe suppression of the growth of the bone marrow. x 166.



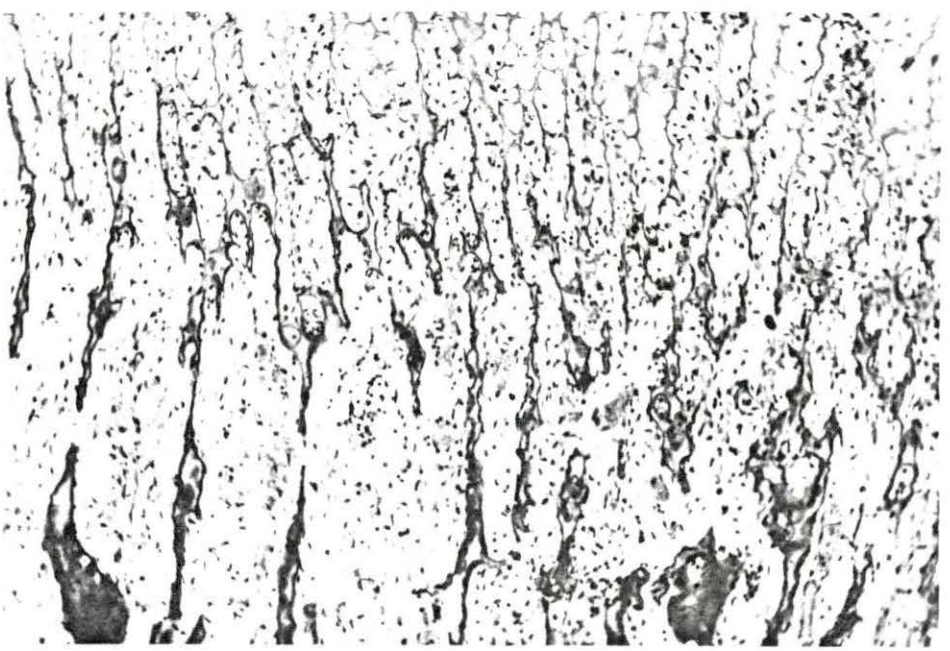
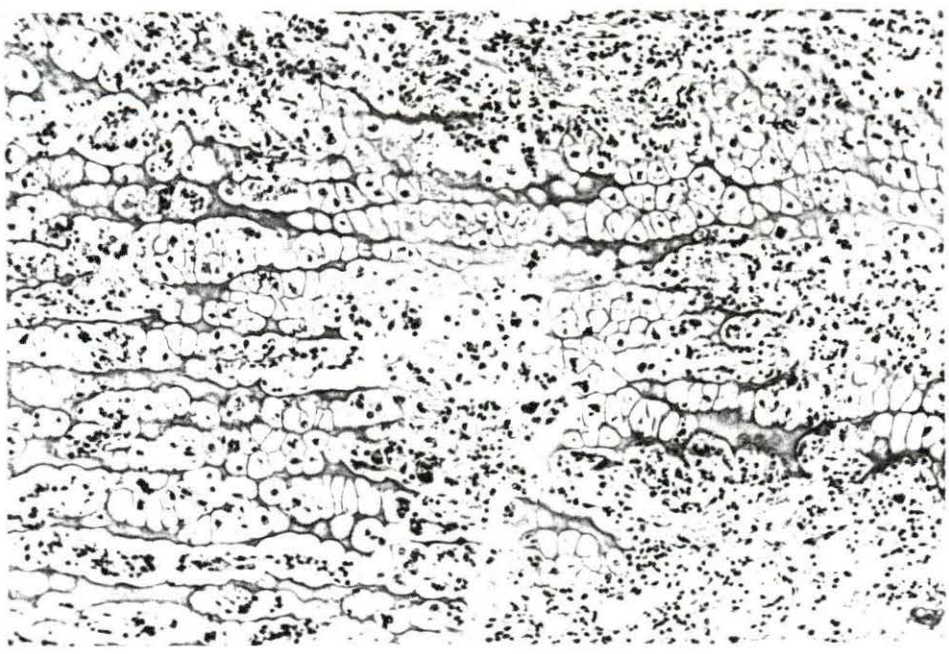


Figure 39. Pig 6(30), age, 16 days: A focal area of necrotic cartilage in the vesicular zone of cartilage undergoing irregular vascular invasion and cartilage destruction. Compare the homogenous appearance of the necrotic cartilage with the adjacent normal cartilage tissues. x 166.

Figure 40. Pig 2(7), age, 6 days: A focal area of necrotic cartilage in the vesicular zone of cartilage undergoing irregular vascular invasion and cartilage destruction. Note homogenous appearance of the necrotic cartilage and the central area of serous inflammation. x 166.



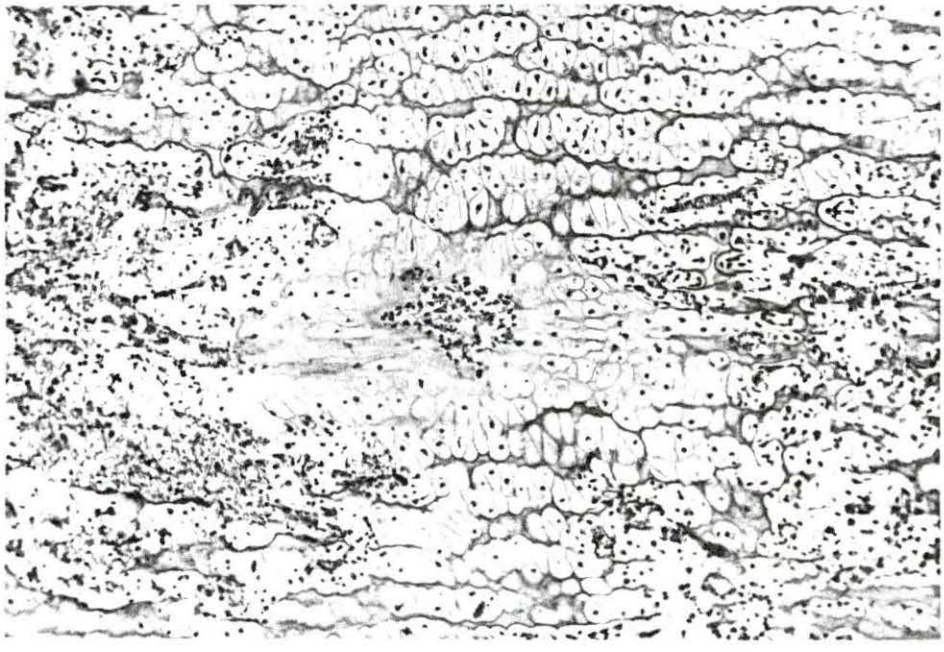


Figure 41. Pig 5(9), age, 12 days: A focal area of necrotic cartilage in the vesicular zone of cartilage. Note the homogenous appearance of the intercellular cartilage matrix and the absence of clearly defined lacunae containing chondrocytes. 714 x.

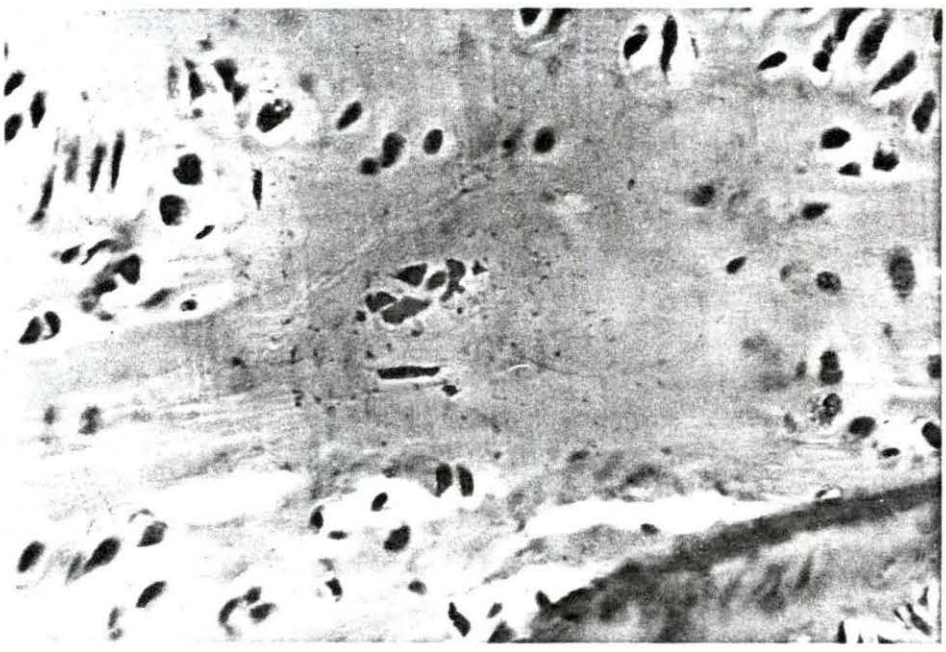
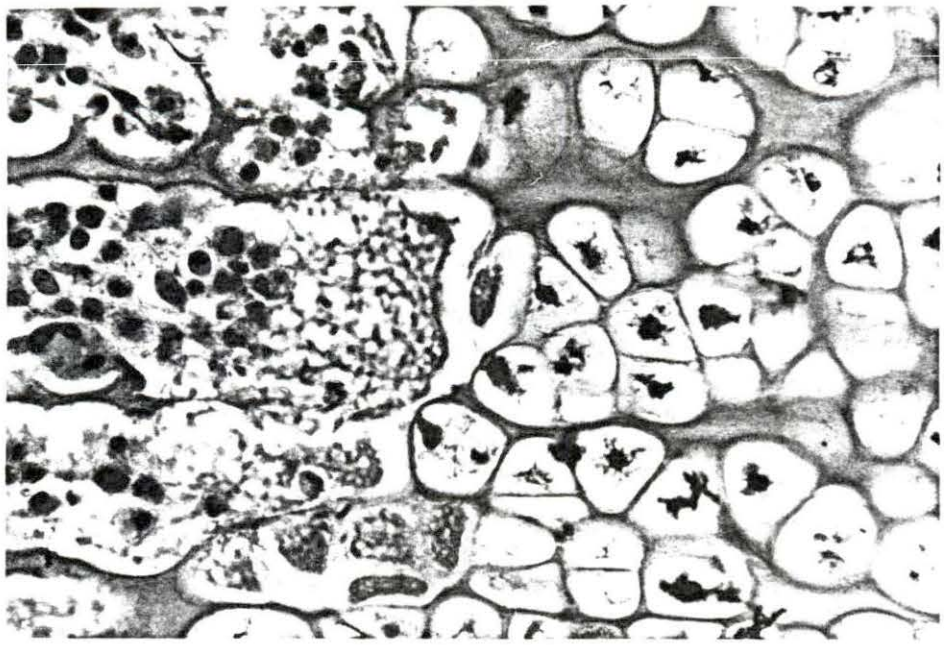


Figure 42. Pig 3(30), age, 4 days: Serous inflammation of the vesicular zone of cartilage at the epiphyseal-metaphyseal junction. Note the network of thick protein strands, the numerous mononuclear phagocytes and the absence of capillary buds, endothelial cells and osteoblasts. x 714.

Figure 43. Pig 3(30), age, 4 days: A large multinucleate syncytial cell in the zone of vascular invasion and cartilage destruction. Note the absence of active cuboidal shaped osteoblasts, the absence of active vascular invasion by capillary buds and the presence of mononuclear phagocytes. x 714.



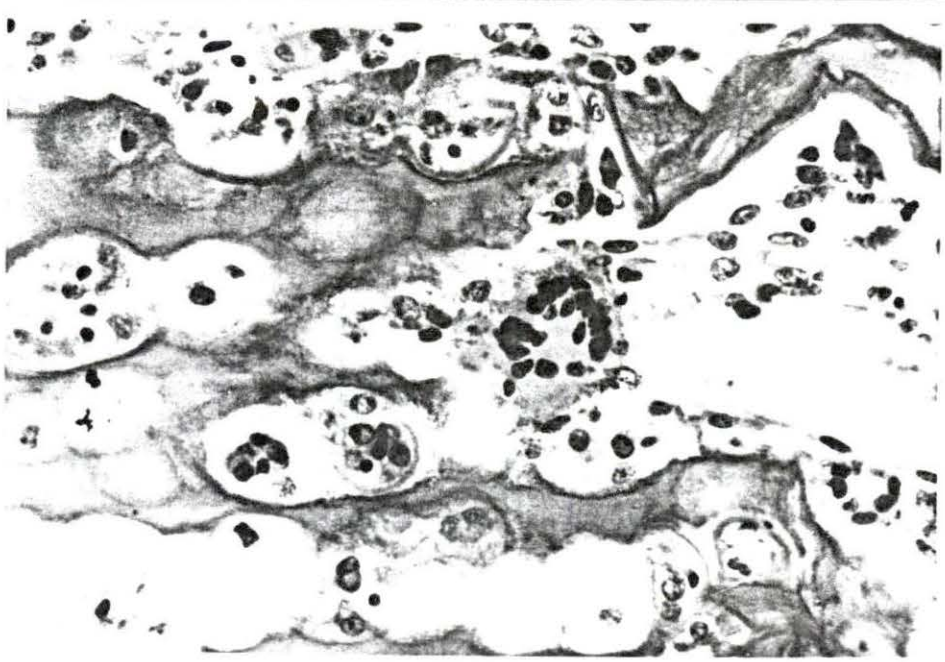
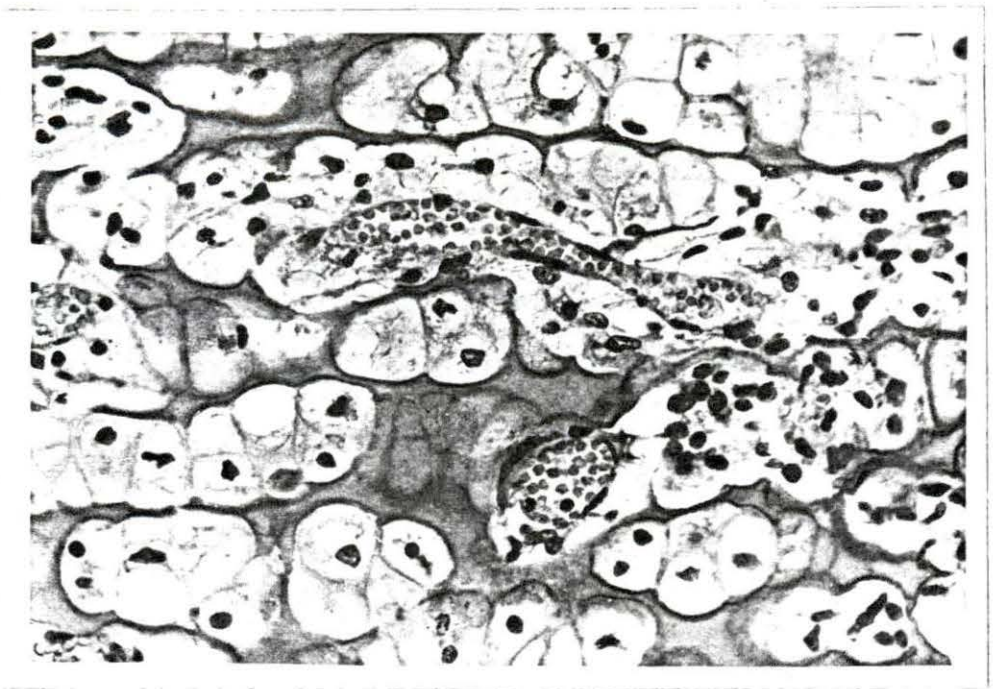


Figure 44. Pig 4(3), age, 10 days: Passively congested capillaries in the vesicular zone of cartilage. Note the absence active cuboidal shaped osteoblasts, the deficient deposition of bone and the presence of numerous mononuclear phagocytes. x 714.

Figure 45. Pig 1(9), age, 20 days: Serous inflammation and edema of the intertrabecular reticular connective tissue in the zone of vascular invasion and cartilage destruction. Note the absence of active cuboidal shaped osteoblasts and the presence of numerous fibroblastic cells and the homogenous appearance of the edematous tissues. x 714.



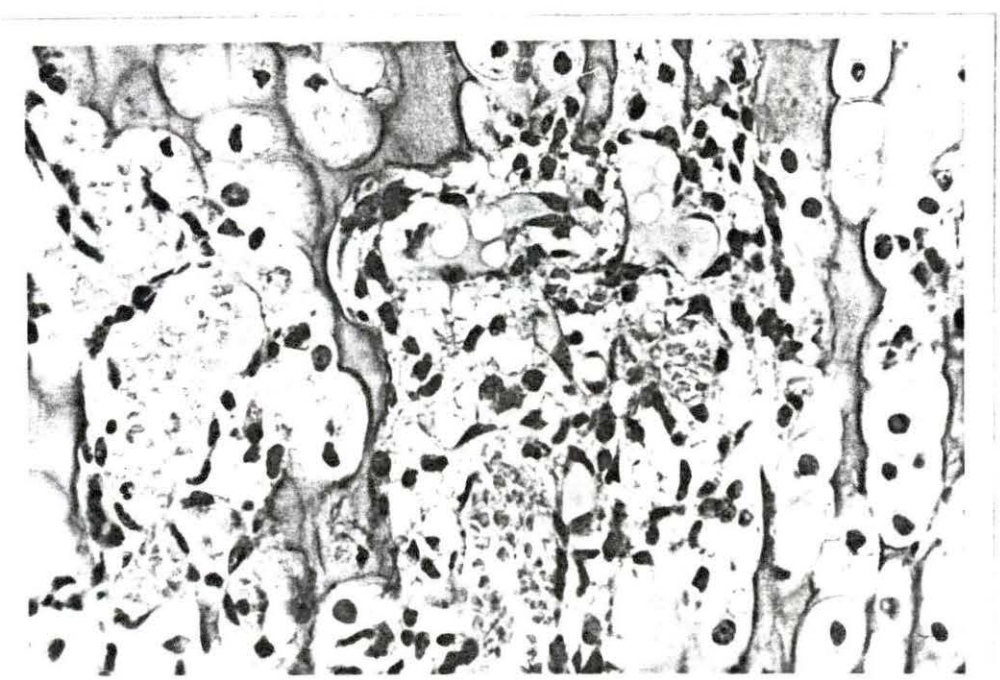
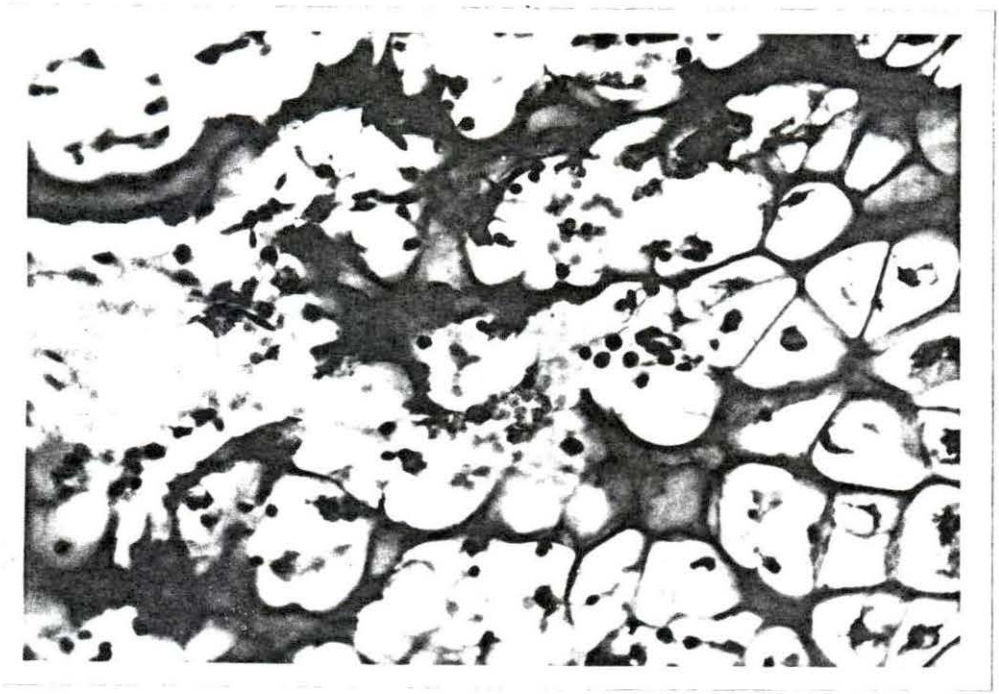


Figure 46. Pig 6(30), age, 16 days: Severe suppression of osteoblasts, endothelial cells and capillary buds. Note the absence of bone deposition and the irregularity in cartilage destruction. x 714.

Figure 47. Pig 1(3), age, 63 days: Numerous active cuboidal shaped osteoblasts bordering the osteochondral trabeculae of the primary spongiosa. Note the delicate reticular intertrabecular connective tissue. x 714.



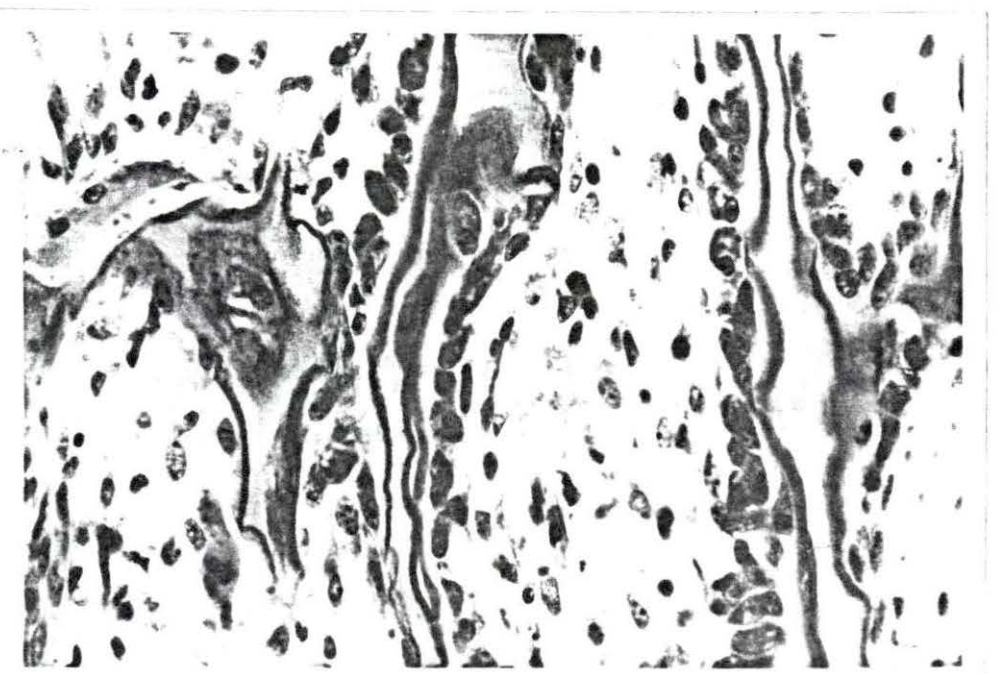
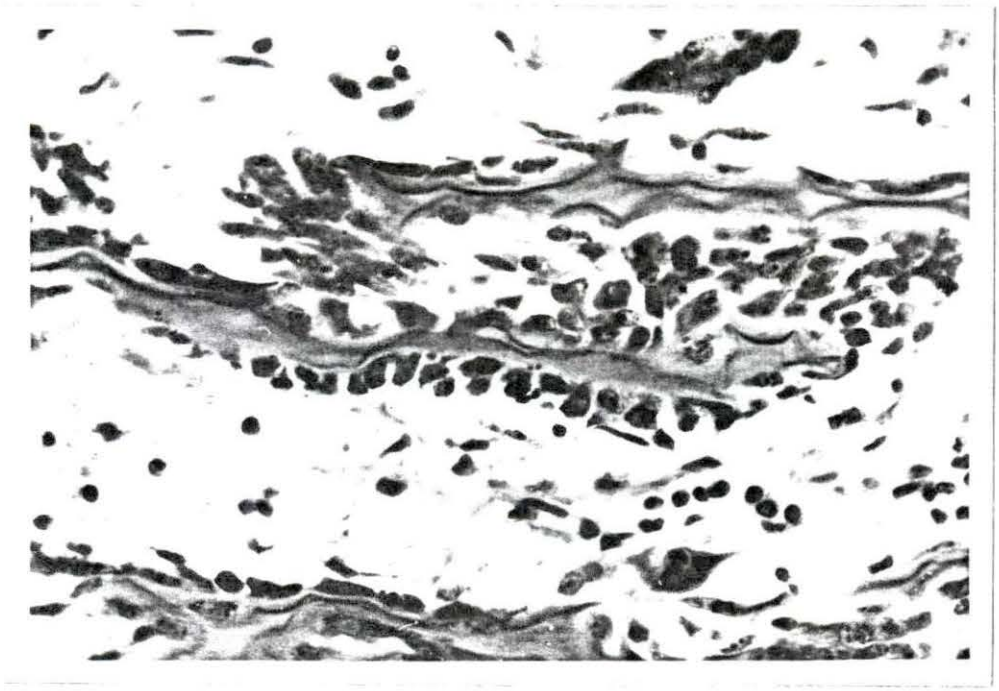


Figure 48. Pig 3(7), age, 42 days: Numerous active cuboidal shaped osteoblasts bordering thin osteochondral trabeculae in the metaphysis. x 714.

Figure 49. Pig 2(9), age, 13 days: Severe suppression of the osteoblasts bordering thin osteochondral trabeculae in the metaphysis. Note the inactive squamous shaped osteoblasts. x 714.



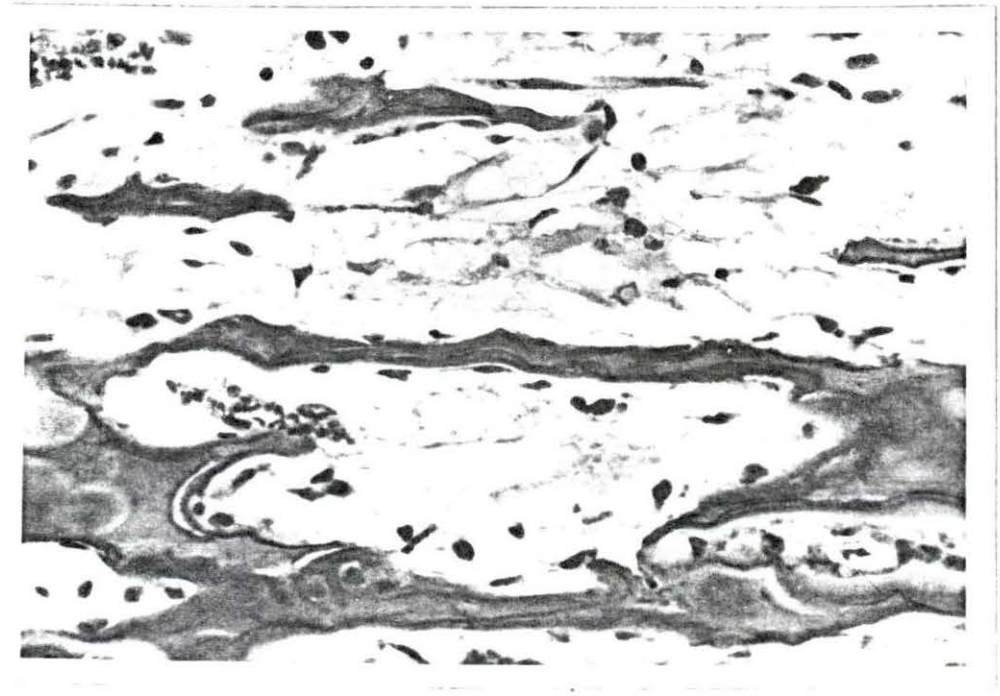
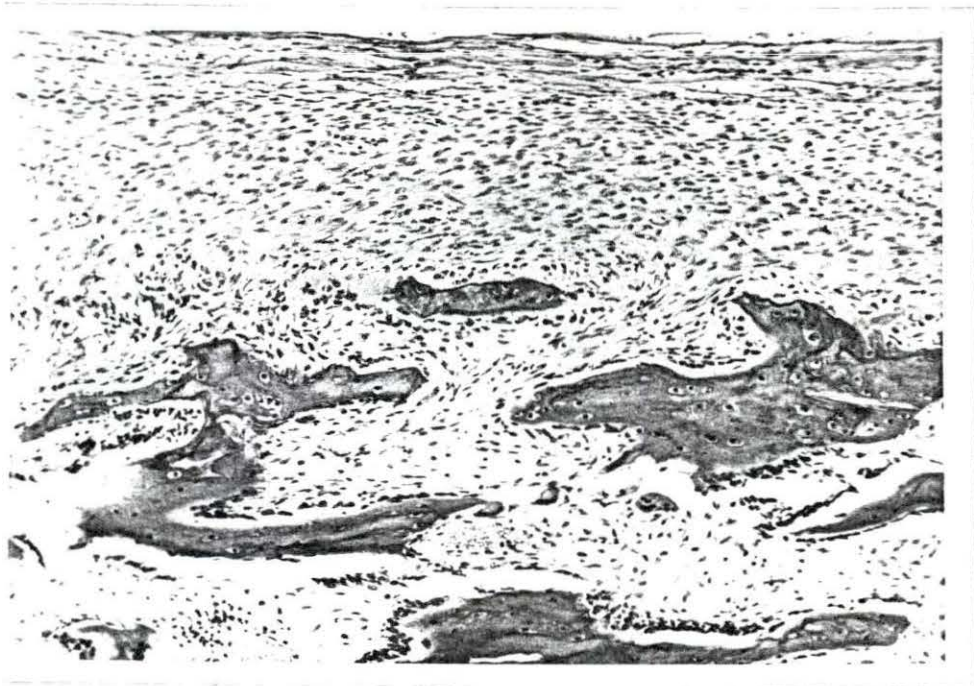


Figure 50. Pig 3(7), age, 42 days: A thick active periosteum with numerous fibroblasts and numerous active cuboidal shaped osteoblasts bordering the trabeculae of the cortex. Note the thick layer of osteoid tissue deposited on the periosteal surface of the small trabecula indicated by the arrow. x 166. (Same microscopic field as Figure 51.)

Figure 51. Pig 3(7), age, 42 days: A thick layer of osteoid tissue on the periosteal surface of a small trabeculae in the cortex. Note the active cuboidal shaped osteoblasts on both the periosteal and medullary surfaces. x 714. (Same microscopic field as Figure 50.)



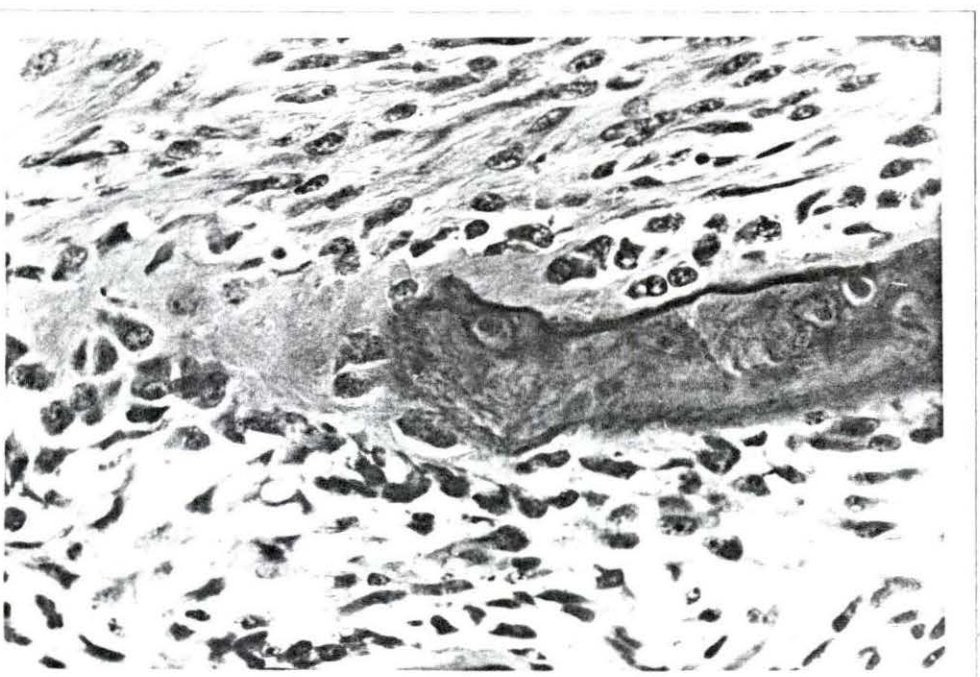
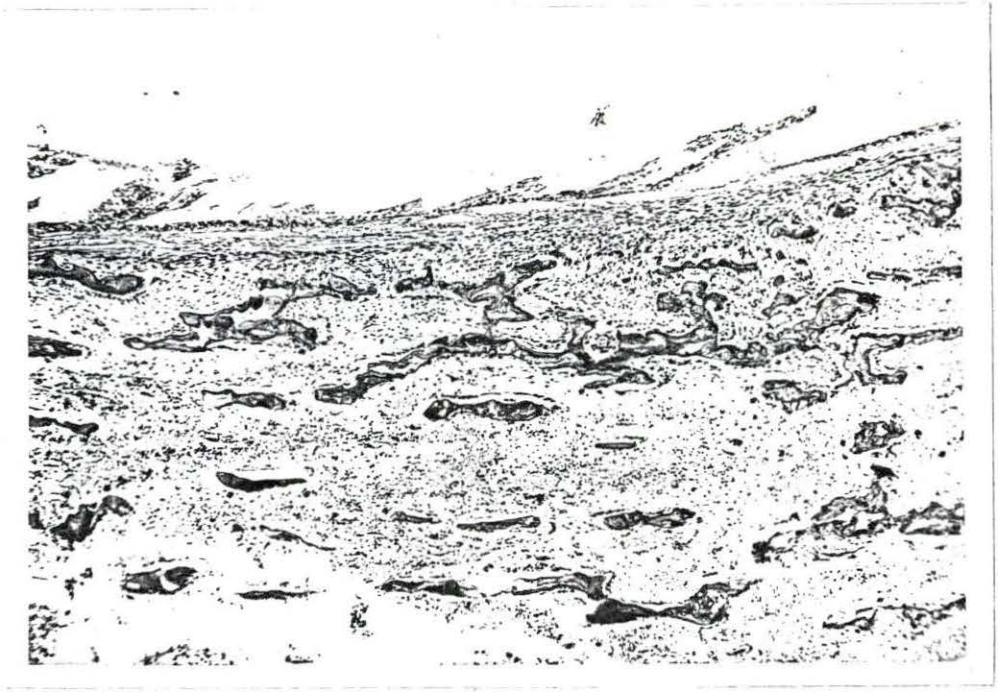


Figure 52. Pig 3(30), age, 4 days: A cortex composed of thick irregularly shaped and sized trabeculae. Note the thin periosteum, the suppression of the growth of the bone marrow and the thick layer of osteoid tissue deposited on the periosteal surface of a trabecula in the cortex. x 66. (Same microscopic field as Figures 53 and 54.)

Figure 53. Pig 3(30), age, 4 days: A cortex composed of thick irregularly shaped and sized trabeculae. Note the thin active periosteum with a few fibroblasts and a moderate number of active cuboidal shaped osteoblasts. Note also the thick layer of osteoid tissue deposited on the periosteal surface of a trabecula of the cortex. x 166. (Same microscopic field as Figures 52 and 54.)



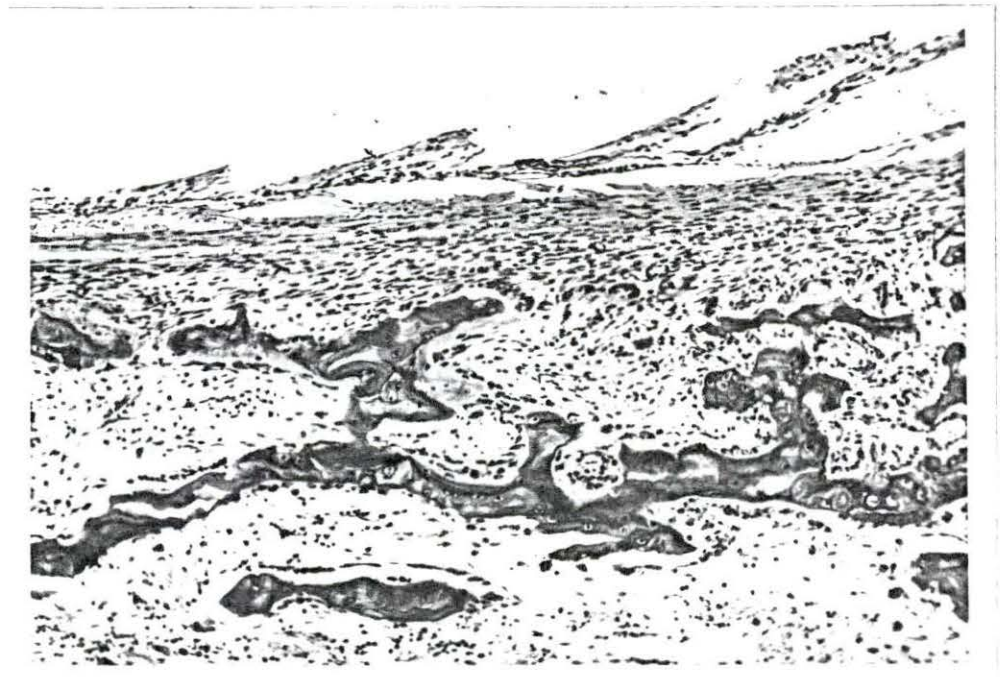
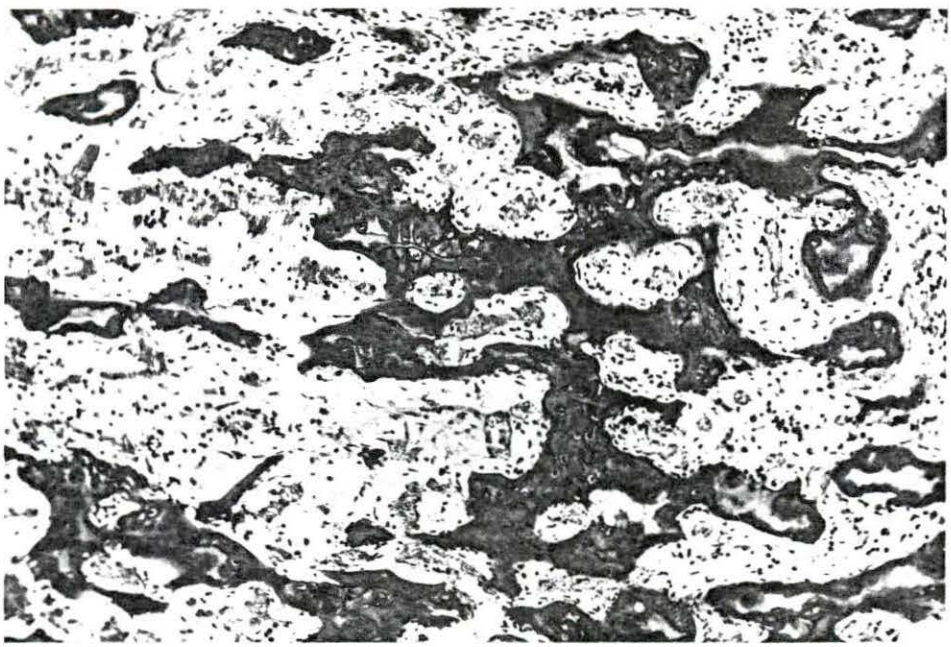


Figure 54. Pig 3(30), age, 4 days: A trabecula of the cortex with a thick layer of osteoid tissue deposited on the periosteal surface and a moderate number of active cuboidal shaped osteoblasts. x 714. (Same microscopic field as Figures 52 and 53.)

Figure 55. Pig 5(30), age, 11 days: Thick trabeculae of various sizes and shapes near the cortical surface of the diaphysis. Note the absence of active cuboidal osteoblasts and the deficient deposition of bone and osteoid tissue. x 166.

Figure 56. Pig 4(3), age, 10 days: Thick trabeculae of various sizes and shapes in the secondary spongiosa of the metaphysis. x 166. (Same microscopic field as Figure 57.)

Figure 57. Pig 4(3), age, 10 days: A thick irregularly shaped small trabecula in the secondary spongiosa. Note the thin layer of osteoid tissue on the surface of this trabecula, the dense fibrillar bone, the central core of intercellular cartilage matrix and the absence of active cuboidal shaped osteoblasts. x 714. (Same microscopic field as Figure 56.)



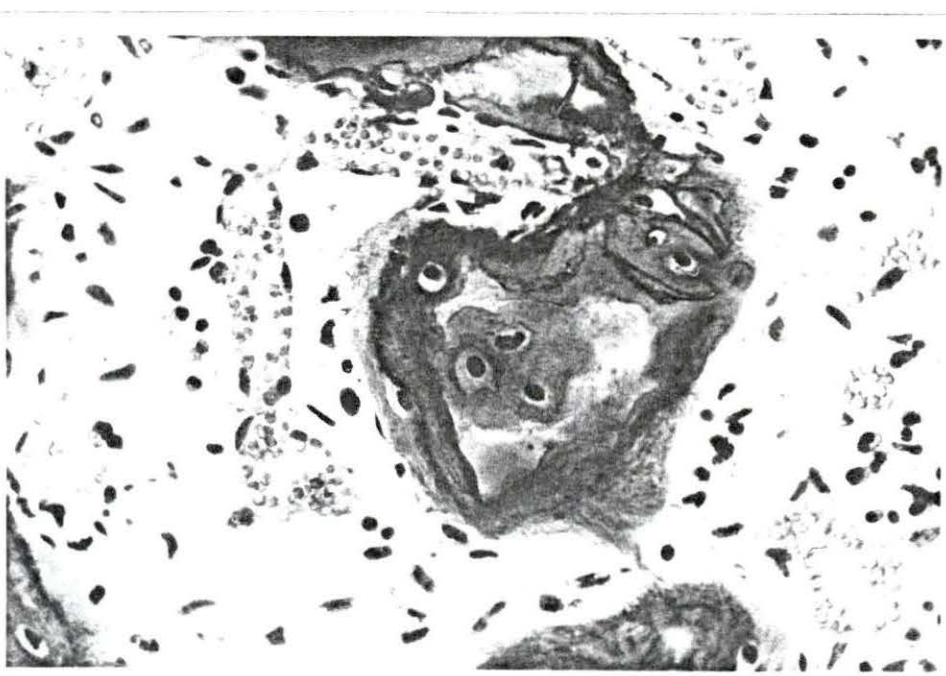
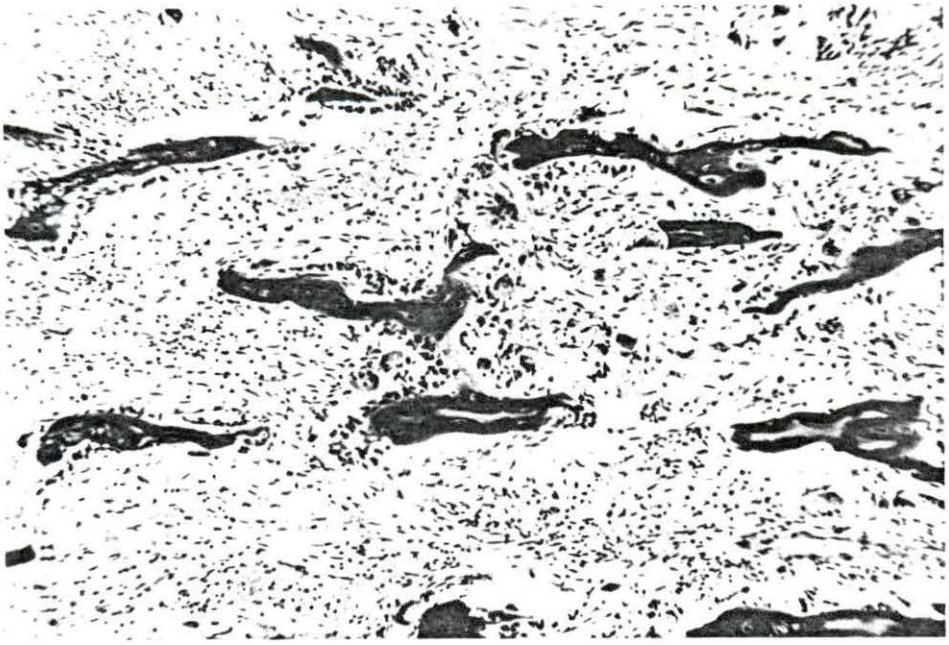


Figure 58. Pig 3(3), age, 16 days: A few scattered trabeculae of irregular sizes and shapes in the secondary spongiosa. Note the irregular distribution of the cuboidal shaped osteoblasts, the numerous multinucleate syncytial cells in the intertrabecular spaces, the dense intertrabecular, reticular connective tissue and the severe suppression of the growth of the bone marrow. Compare this figure with Pig 3(7), Figure 59. x 166.

Figure 59. Pig 3(7), age, 42 days: Numerous long, thin osteochondral trabeculae in secondary spongiosa. Note the numerous active, cuboidal shaped osteoblasts, the dense reticular intertrabecular connective tissue and the moderated suppression of the growth of the bone marrow. Compare this figure with Pig 3(3), Figure 58. x 166.



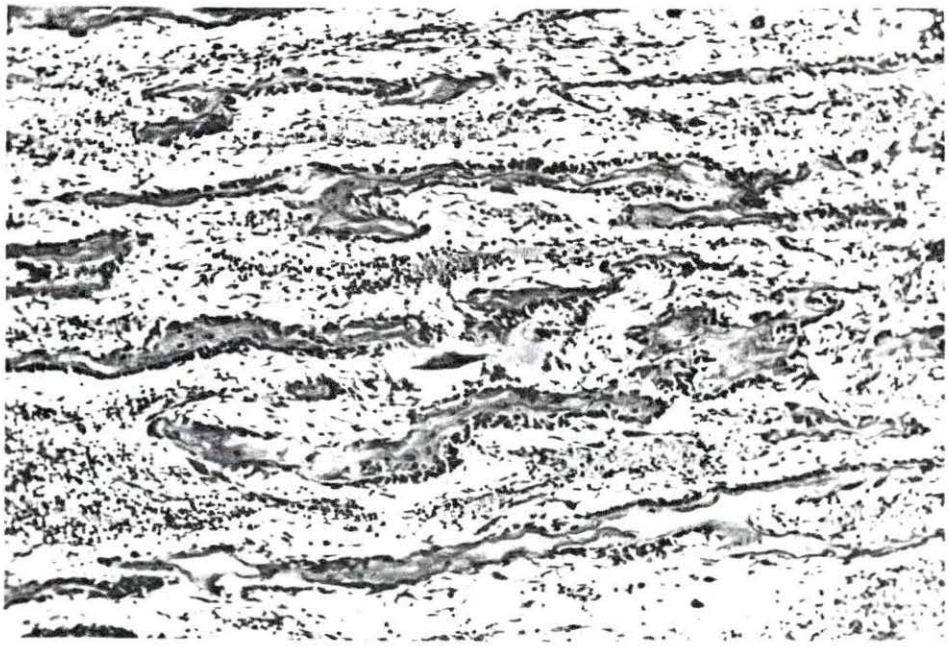
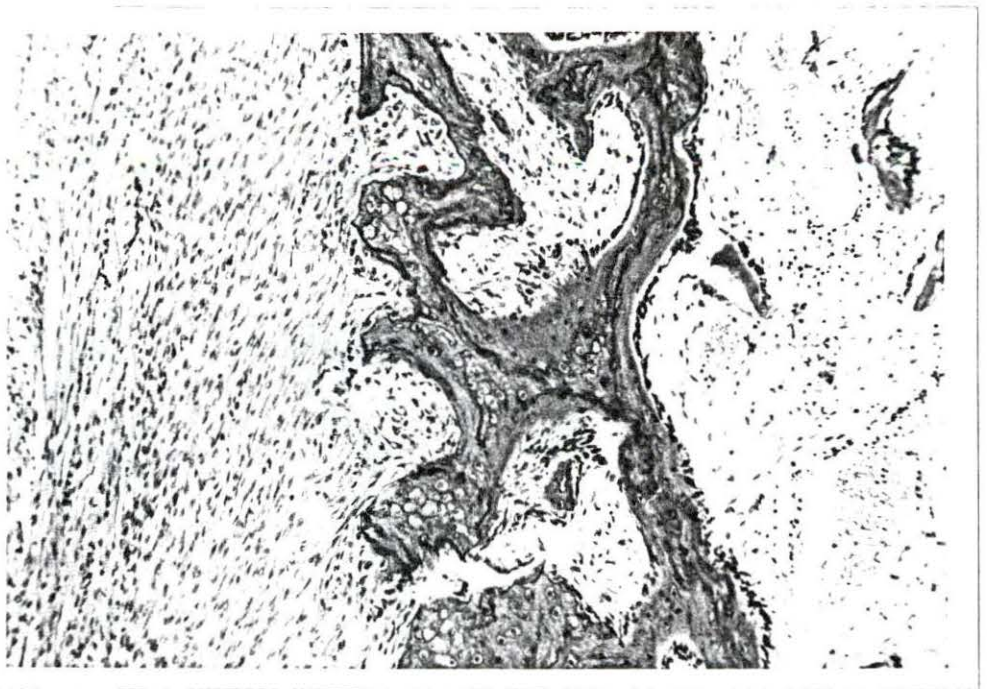


Figure 60. Pig 3(7), age, 42 days: A thick active periosteum with numerous fibroblasts and numerous active cuboidal shaped osteoblasts. The cortex is composed of thick continuous trabeculae which are forming haversian canals. Compare this figure with Pig 5(30), Figure 61. x 166.

Figure 61. Pig 5(30), age, 11 days: A thin inactive cortex with a few fibroblasts and a few inactive squamous shaped osteoblasts. The cortex is formed by thin continuous trabeculae. Note the absence of active cuboidal shaped osteoblasts and the deficient deposition of bone. Compare this figure with Pig 3(7), Figure 60. x 166.



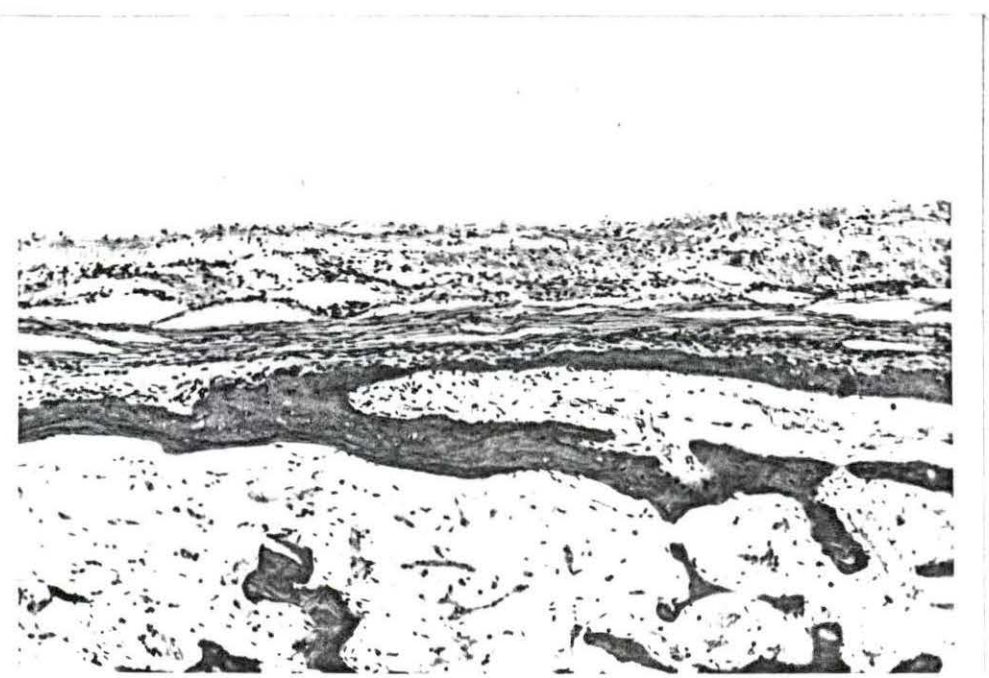
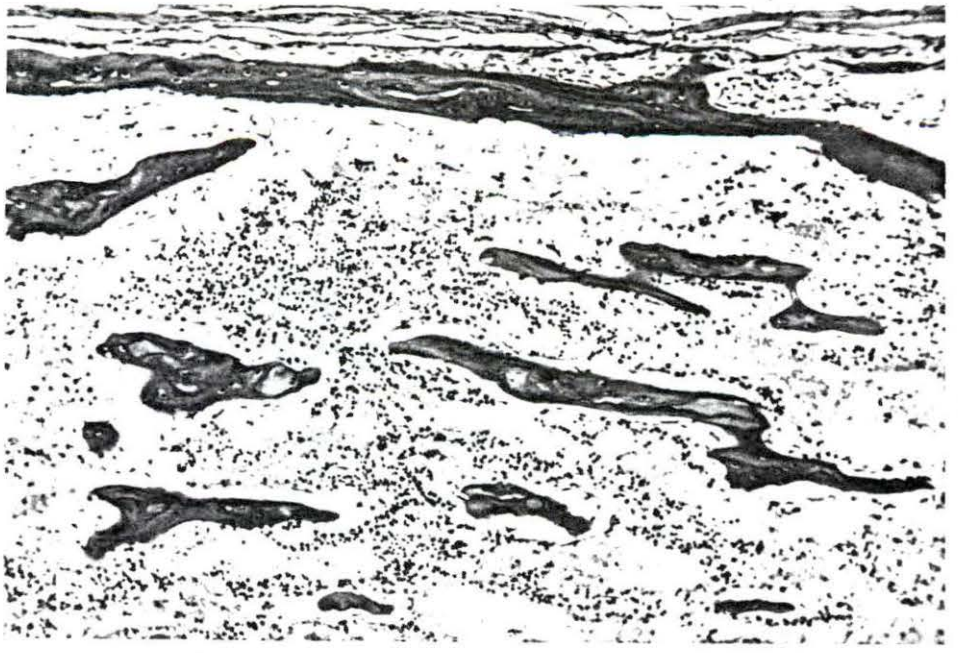


Figure 62. Pig 6(30), age, 16 days: A thin periosteum and cortex with moderate suppression of the growth of the bone marrow. Note the absence of active cuboidal shaped osteoblasts and fibroblasts. Compare this figure with Pig 1(3), Figure 63. x 166.

Figure 63. Pig 1(3), age, 63 days: A thick active periosteum and cortex with numerous active cuboidal shaped osteoblasts and numerous fibroblasts. Note the dense reticular, intertrabecular connective tissue and the absence of bone marrow. Compare this figure with Pig 6(30), Figure 62. x 166.



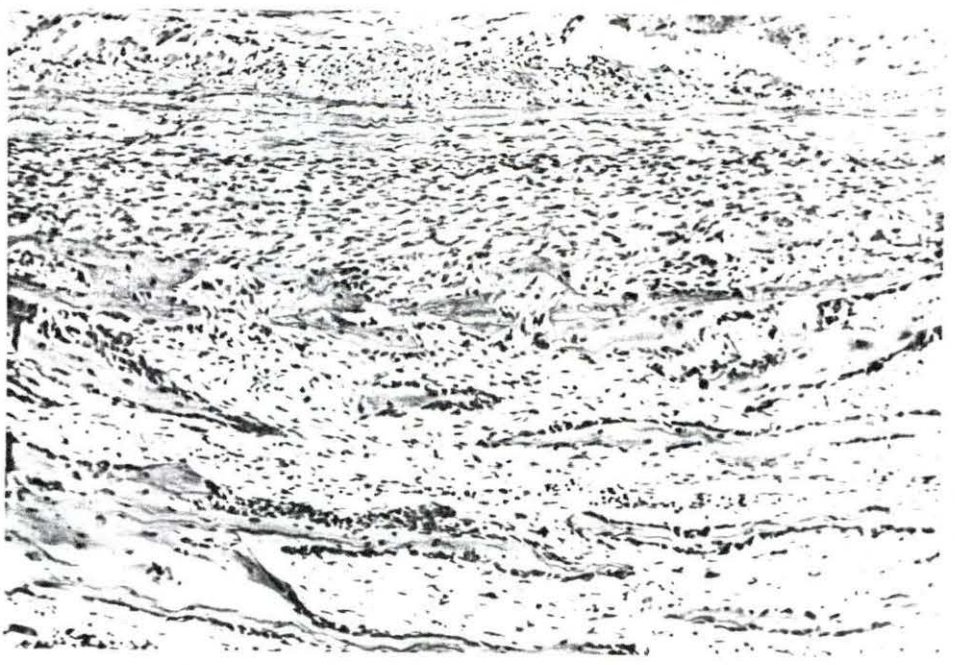
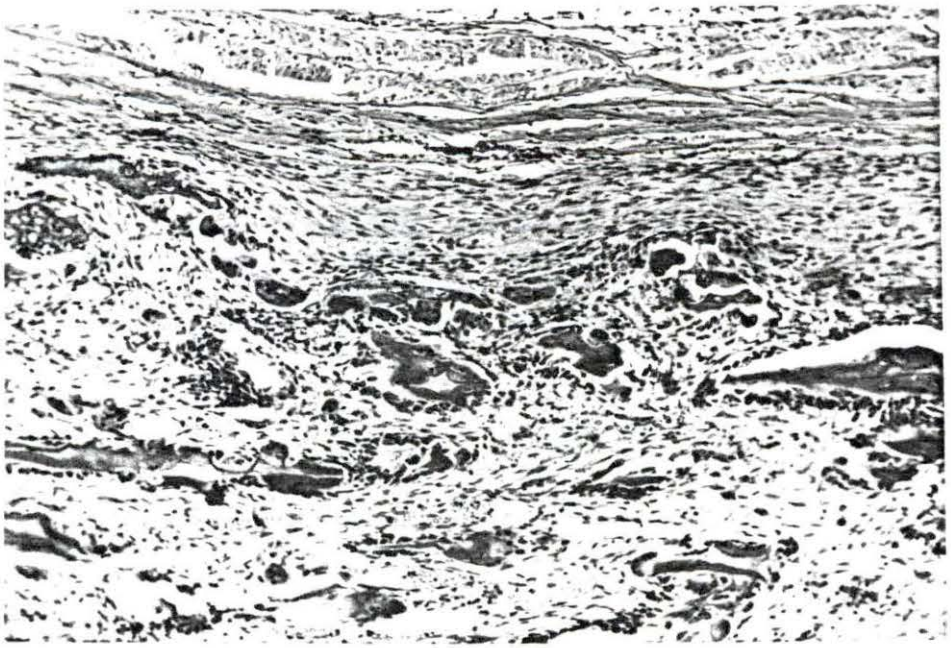


Figure 64. Pig 1(3), age, 63 days: A thick active periosteum with numerous fibroblasts, numerous active cuboidal osteoblasts and numerous large multinucleate syncytial cells near the epiphyseal-diaphyseal junction. Note the irregular distribution of the trabeculae of the cortex and the dense reticular, intertrabecular connective tissue. x 166. (Same microscopic field as Figure 65.)

Figure 65. Pig 1(3), age, 63 days: Numerous multinucleate syncytial cells on the periosteal surface of the trabeculae of the cortex near the epiphyseal-diaphyseal junction. Note the numerous fibroblasts and active cuboidal shaped osteoblasts. x 714. (Same microscopic field as Figure 64.)



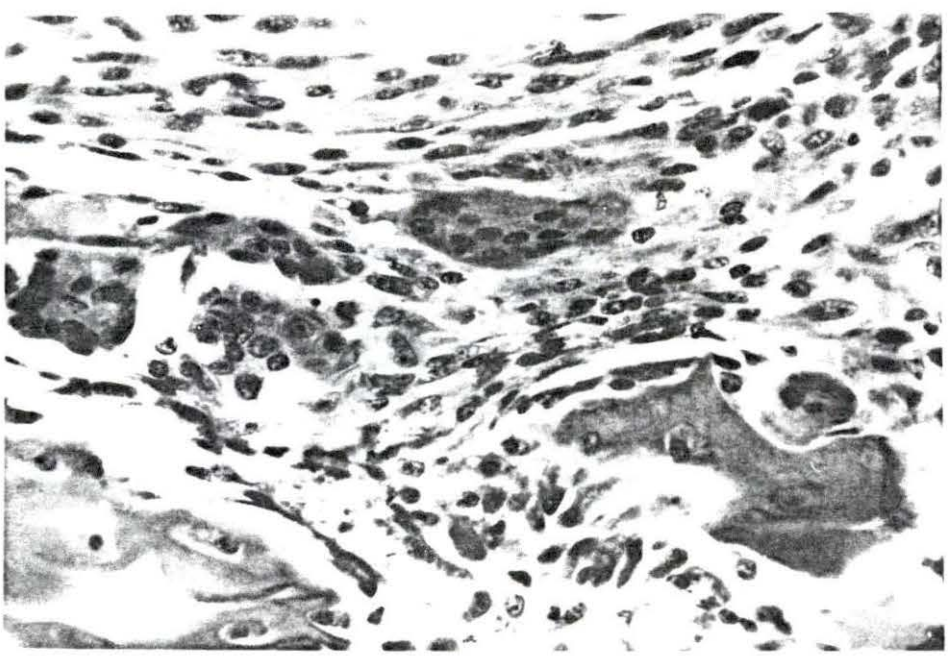
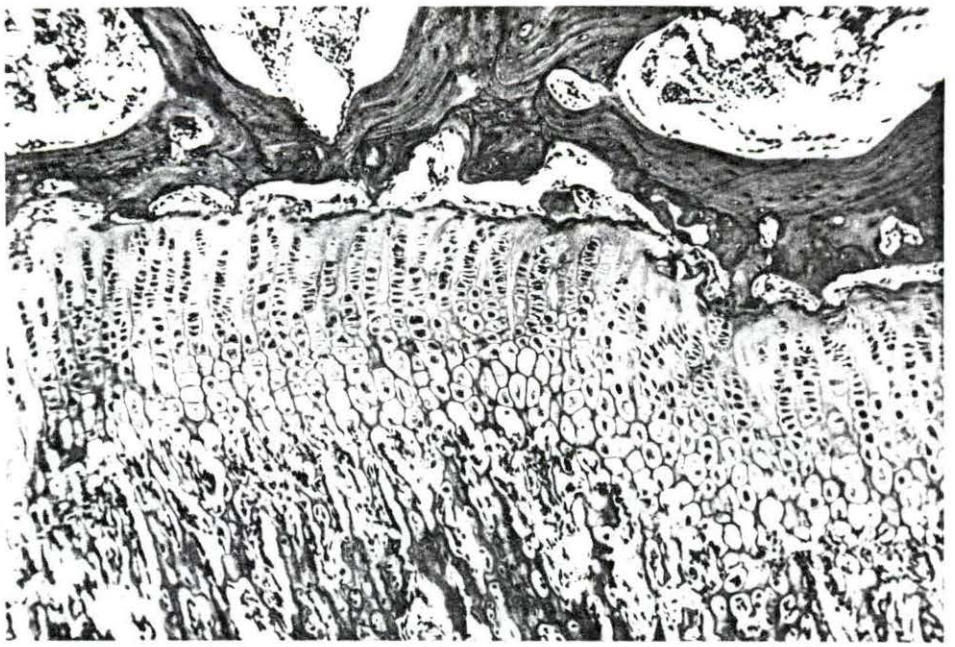


Figure 66. Rat (N-33), age, 55 days: An epiphysis of a normal rat. Note the straight uniform column of chondrocytes in the proliferating and vesicular zones of cartilage. Compare this figure with Rat (N-71), Figure 67. x 166.

Figure 67. Rat (N-71), age, 93 days: An epiphysis of a normal rat. Note the increase in intercellular cartilage matrix and the irregularity in the size and shape of the chondrocytes in the proliferating and vesicular zones. Note the broad zone of large vesicular chondrocytes near the epiphyseal-metaphyseal junction. This is an artifact resulting from a curvature in the epiphyseal plate of cartilage. Compare this figure with Rat (N-33), Figure 66. x 166.



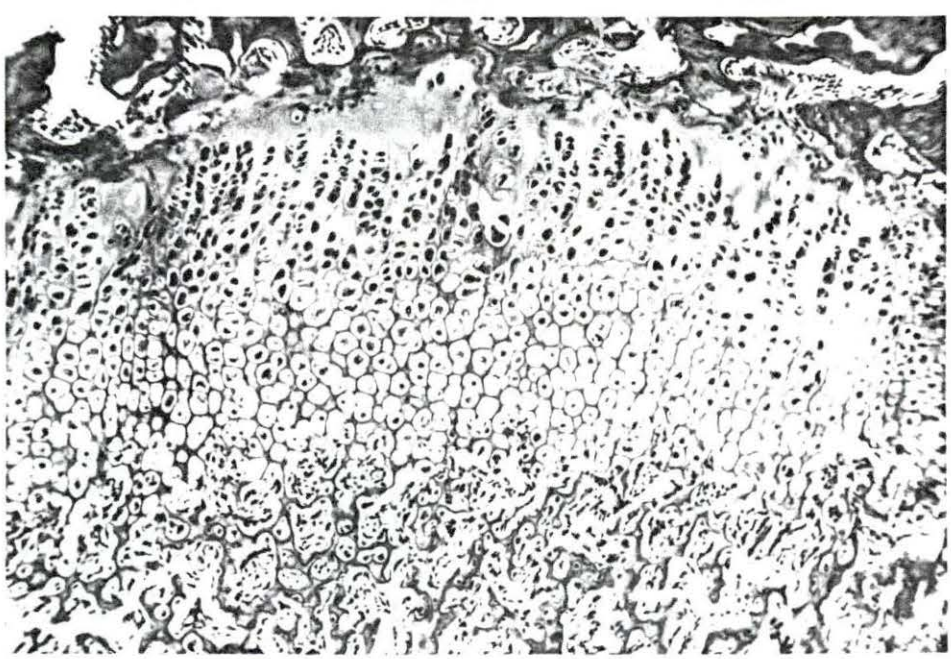
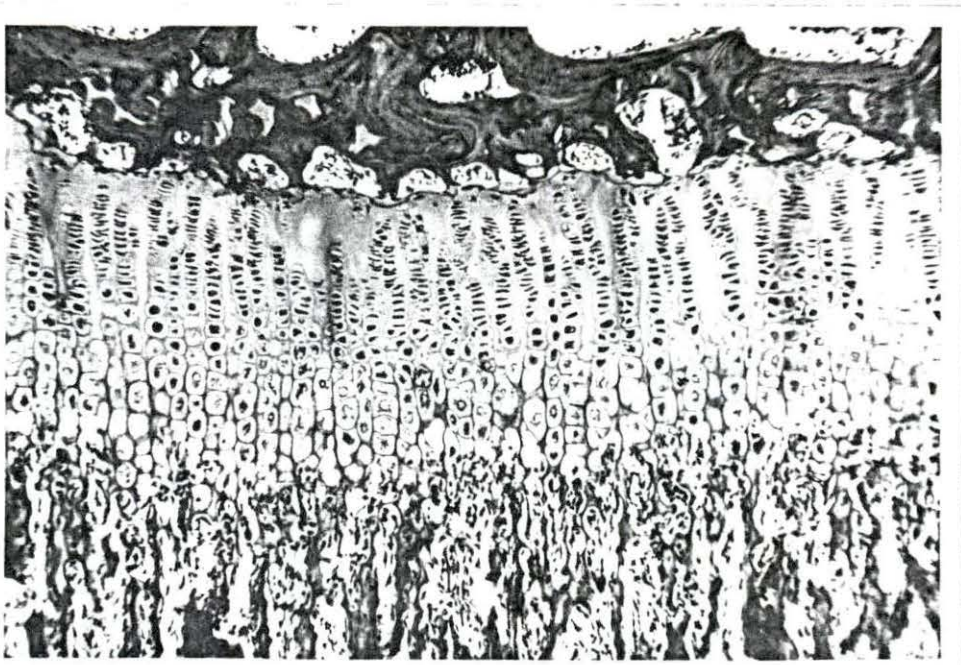


Figure 68. Rat (M-31), age, 53 days: A normal epiphyseal plate of cartilage. Note the straight columns of chondrocytes in the proliferating and vesicular zones of cartilage. Note also the large number of cells, endothelial cells, capillary buds, and osteoblasts, in the zone of vascular invasion and cartilage destruction. Compare this figure with Rat (A-47), Figure 69. x 166.

Figure 69. Rat (A-47), age, 69 days: The epiphyseal plate of cartilage of a rat with brachychondroplasia resulting from a severe deficiency of A vitamins. Note the thin vesicular and proliferating zones of cartilage, the absence of cells in the zone of vascular invasion and cartilage destruction and the deficient deposition of bone. Compare this figure with Rat (M-31), Figure 68. x 166.



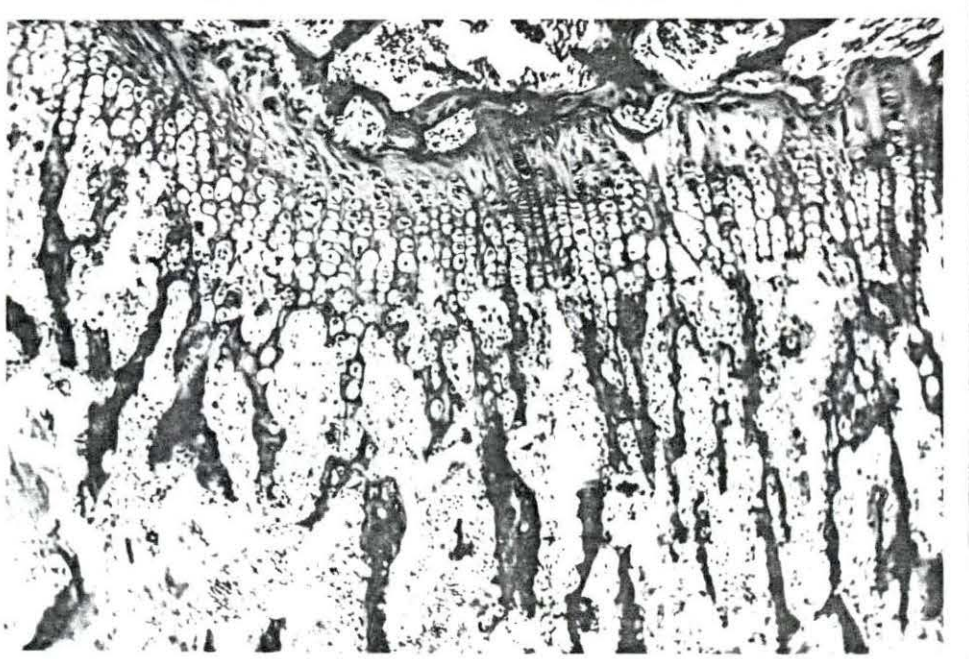
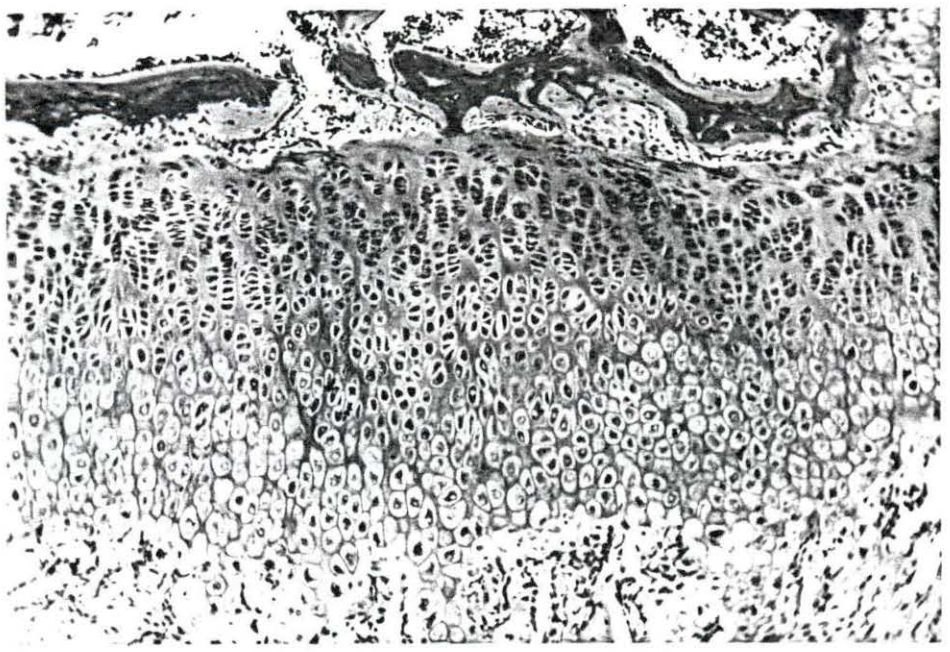


Figure 70. Rat (R-31), age, 53 days: The epiphyseal plate of cartilage of a rat with severe experimental rickets. Note the broad zones of vesicular and proliferating cartilage. Compare this figure with Rat (M-31), Figure 68. x 166.

Figure 71. Rat (R-31), age, 53 days: The epiphyseal plate of cartilage and metaphysis of a rat with severe experimental rickets. Note the irregularity in the vesicular zone of cartilage, the vascular channels and the numerous cells at the epiphyseal-metaphyseal junction. Note also the osteoid trabeculae of the metaphysis containing trapped chondrocytes. Compare this figure with Rat (M-31), Figure 68. x 166.



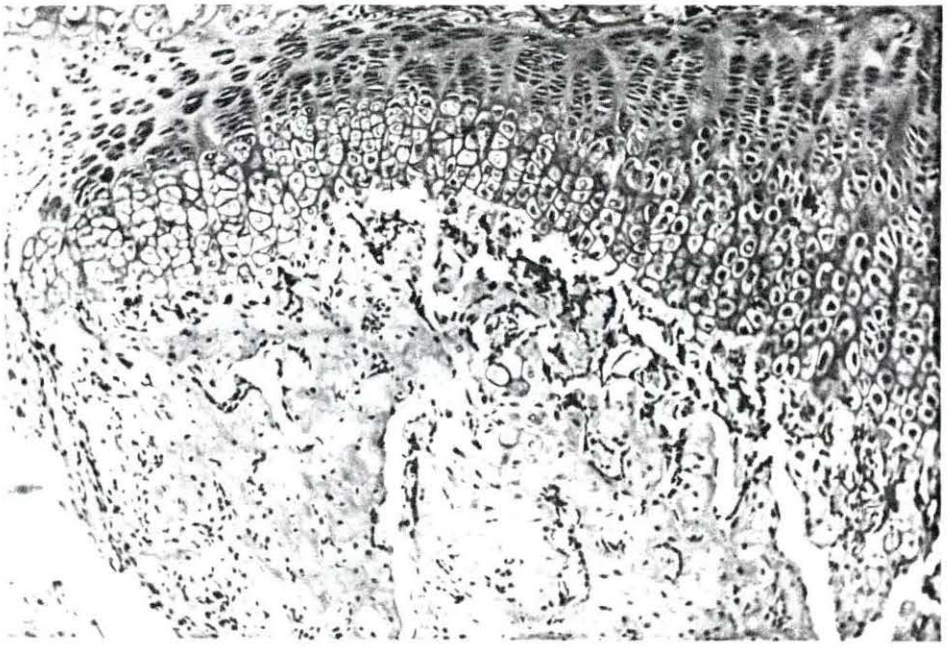
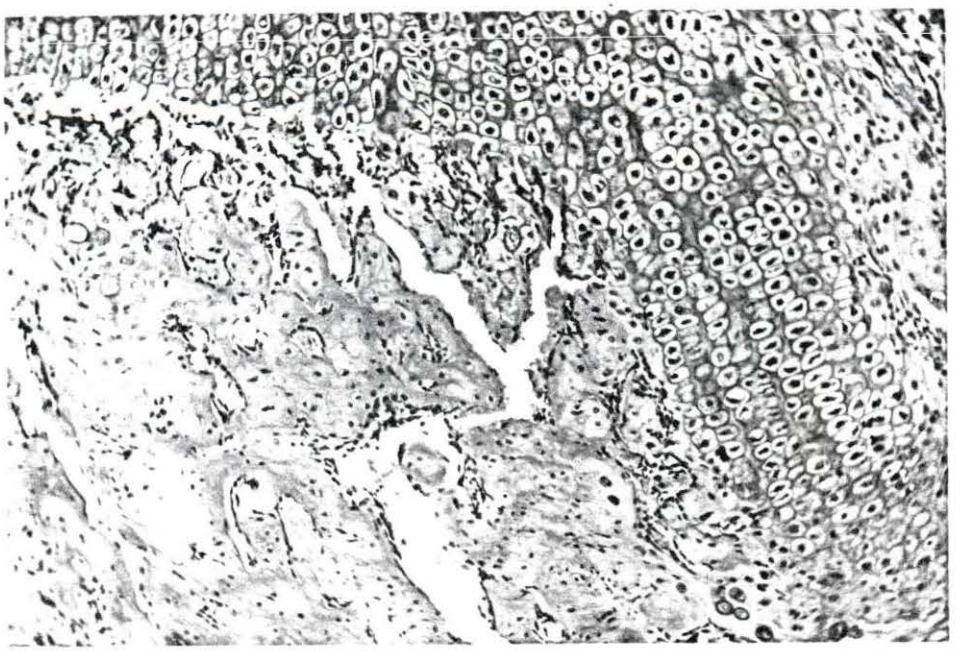


Figure 72. Rat (R-31), age, 53 days: A vascular channel with the characteristic "bush" appearance of classical rickets and experimental rickets. Note the projecting tongue of vesicular cartilage adjacent to the blood vessels. x 166.

Figure 73. Rat (R-31), age, 53 days: A focal area of necrotic cartilage at the epiphyseal-metaphyseal junction of a rat with experimental rickets. Note the nest of chondrocytes showing rejuvenation in the center of the necrotic cartilage. x 166.



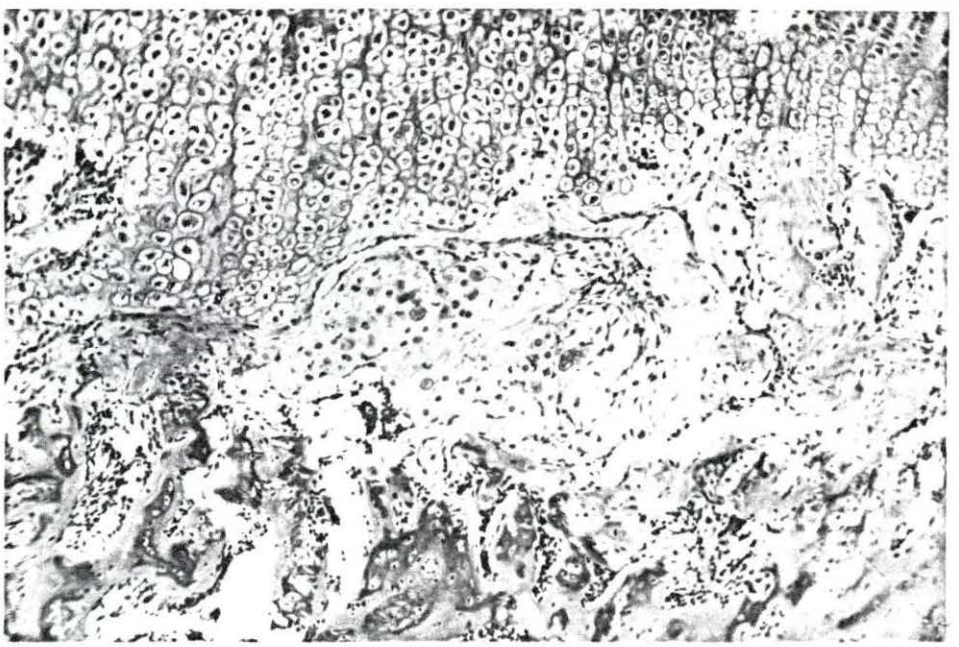


Figure 74. Rat (R-31), age, 53 days: A projecting tongue of vesicular cartilage extending into the metaphysis of a rat with experimental rickets. Note the compression of the chondrocytes and the distortion of the cartilage columns. x 166.

Figure 75. Rat (R-31), age, 53 days: Two projecting tongues of vesicular cartilage extending into the metaphysis of a rat with experimental rickets. Note the severe distortion of the cartilage columns and the thick layers of osteoid tissue deposited on the surface of the matrix of cartilage. x 166.

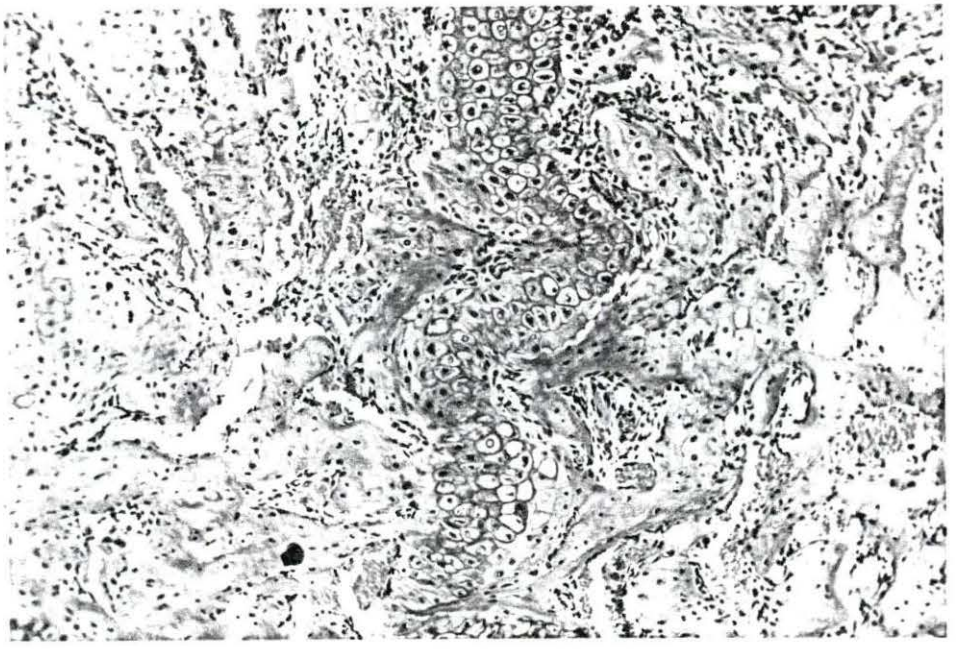
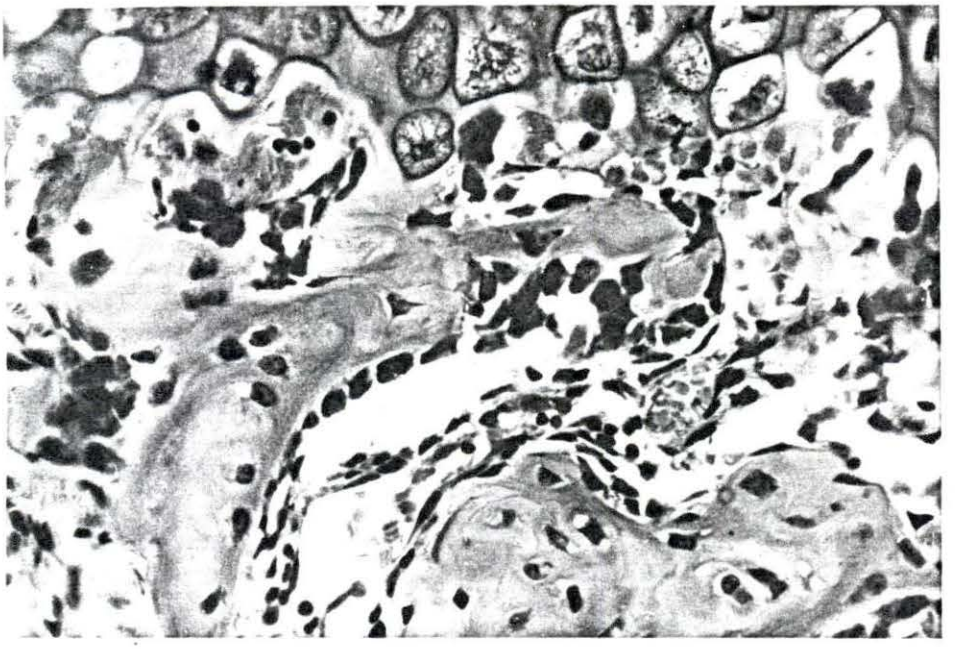


Figure 76. Rat (R-31), age, 53 days: Active cuboidal shaped osteoblasts bordering a vascular channel of a rat with experimental rickets. Note the thick layers of osteoid tissue contain immature osteocytes. Compare this figure with Rat (A-47), Figure 77. x 714.

Figure 77. Rat (A-47), age, 69 days: Inactive squamous shaped osteoblasts bordering osteotrabeculae of the metaphysis of a rat with a severe deficiency of A vitamins. Note the deficient deposition of bone and the absence of endothelial cells. x 714.



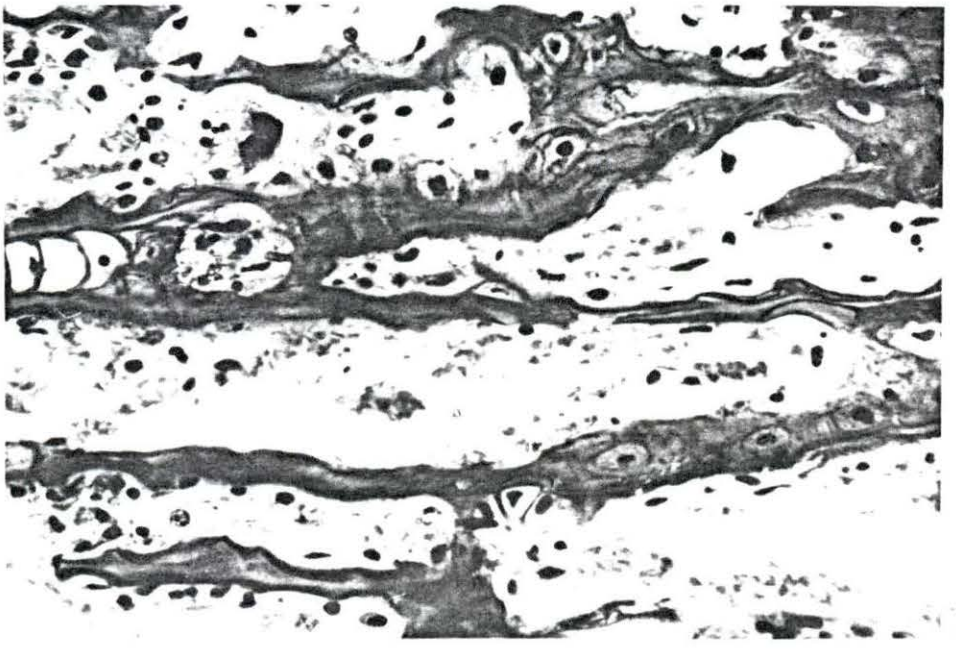


Figure 78. Rat (M-42), age, 64 days: Active cuboidal shaped osteoblasts bordering osteochondral trabeculae in the metaphysis of a rat fed the experimental diet consumed by the pigs during this experiment, Ration A. Note the number of osteoblasts, endothelial cells and mononuclear phagocytes. Compare this figure with Rat (A-47), Figure 77. x 714.

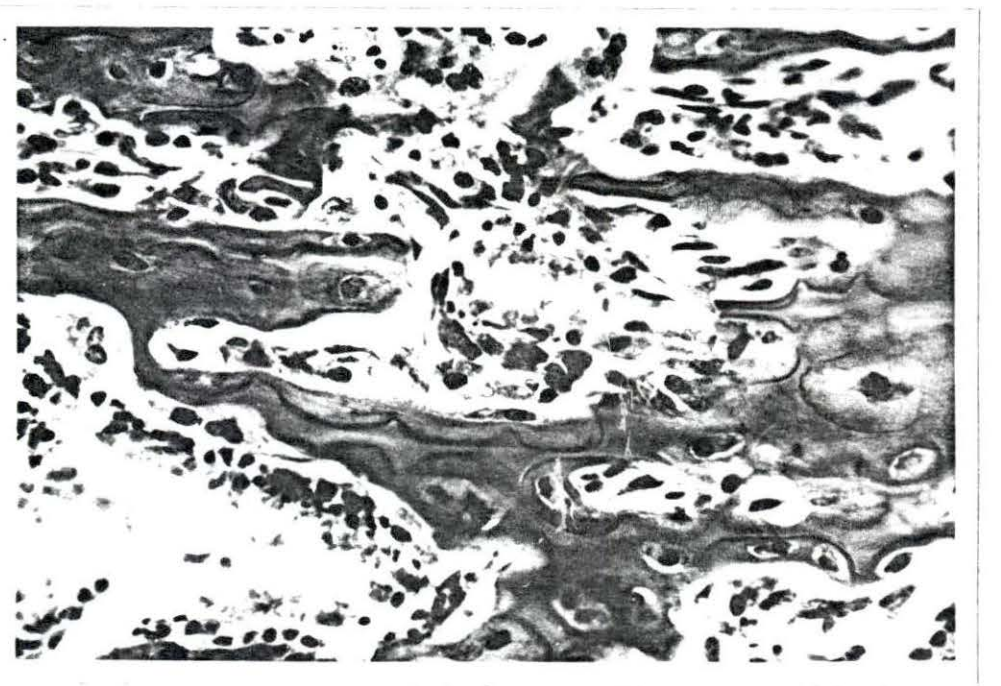
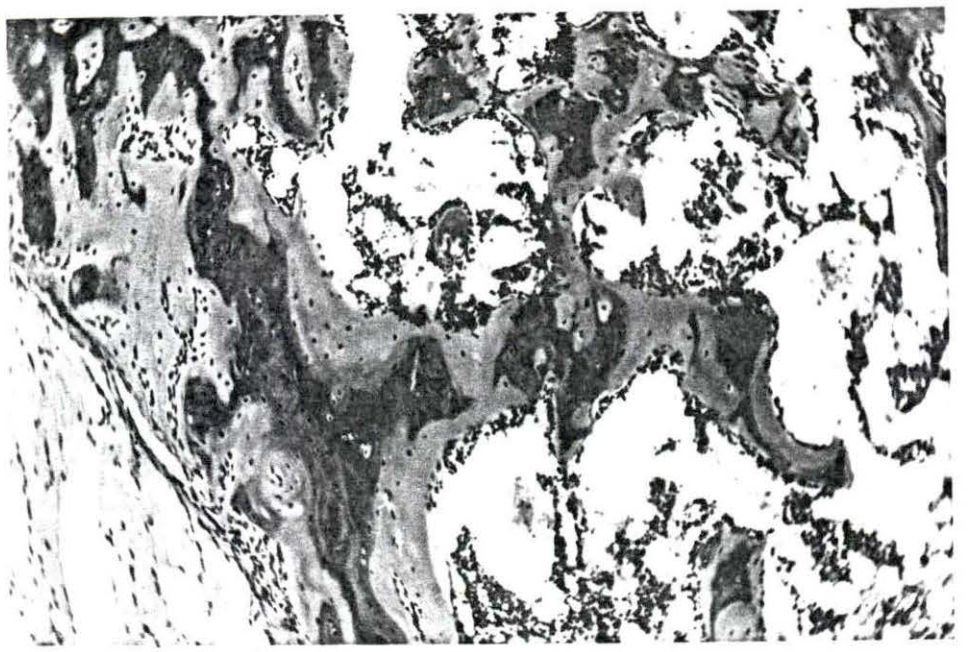


Figure 79. Rat (R-31), age, 53 days: The cortex of a rat with severe experimental rickets. Note the thick layers of osteoid deposited on the osteochondral trabeculae. Compare this figure with Rat (N-33), Figure 83. x 166.

Figure 80. Rat (R-31), age, 53 days: Thick osteoid tissue layers deposited in the haversian canal of the cortex. Note the fibrillar appearance of the osteoid tissue and the difference in the density of newly deposited bone and the old bone. x 714.



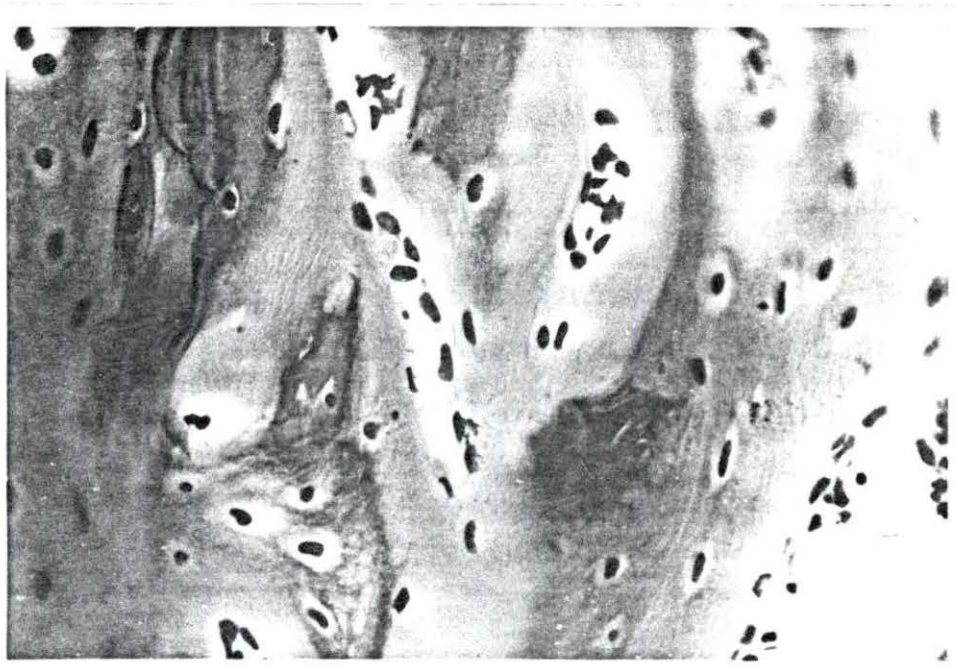
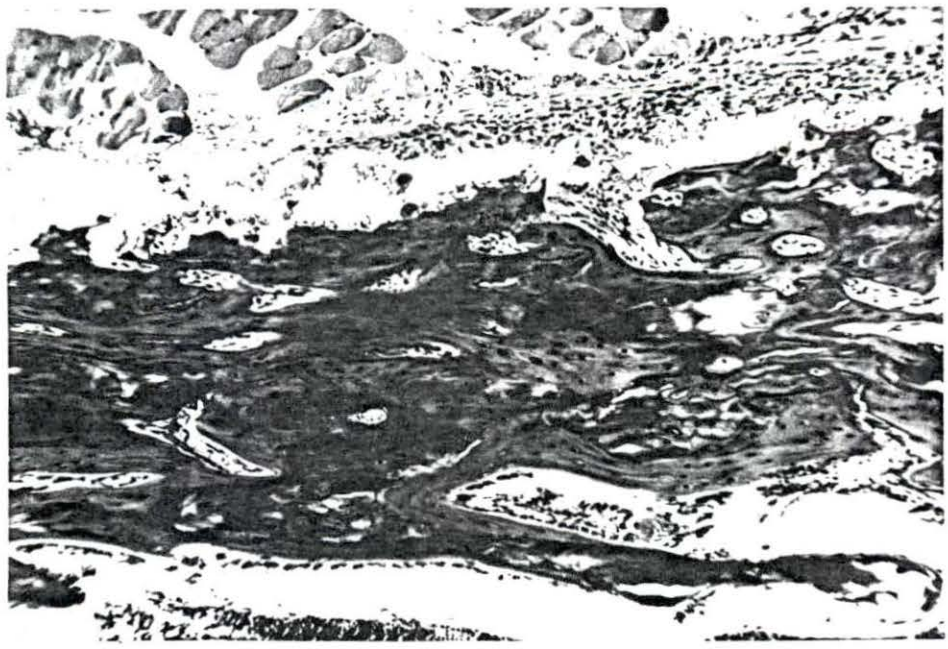


Figure 81. Rat (M-42), age, 64 days: A normal cortex near the epiphyseal-diaphyseal junction of a rat fed Ration A. Note the thin periosteum and the large size of the haversian canals. Compare this figure with Rat (M-71), Figure 82. x 166.

Figure 82. Rat (M-71), age, 93 days: A normal thick cortex with small haversian canals. Note the thin periosteum and the difference in the density of the newly deposited bone and the old bone of the osteochondral trabeculae. Compare this figure with Rat (M-42), Figure 81. x 166.



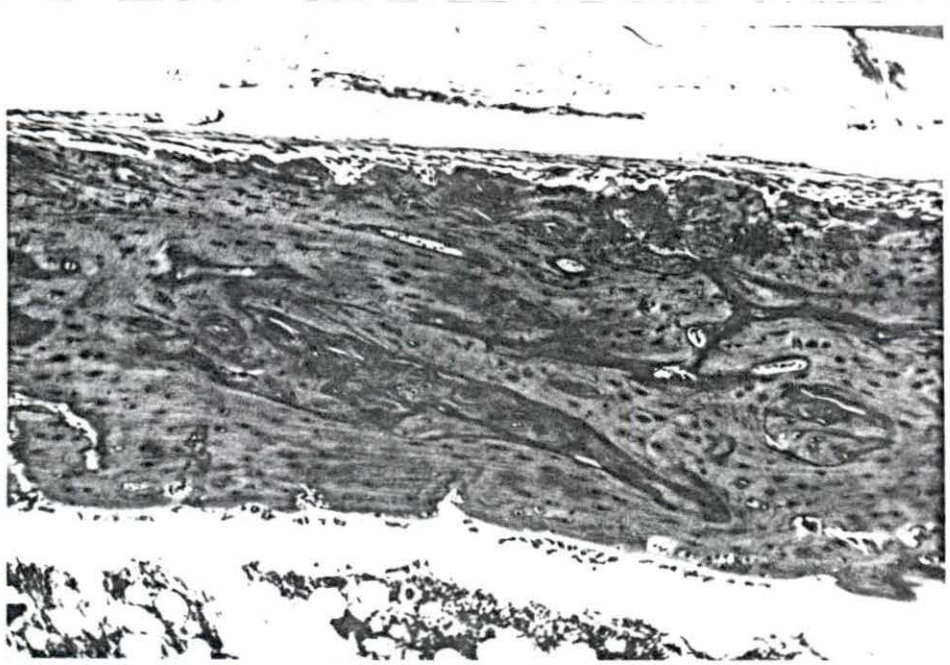
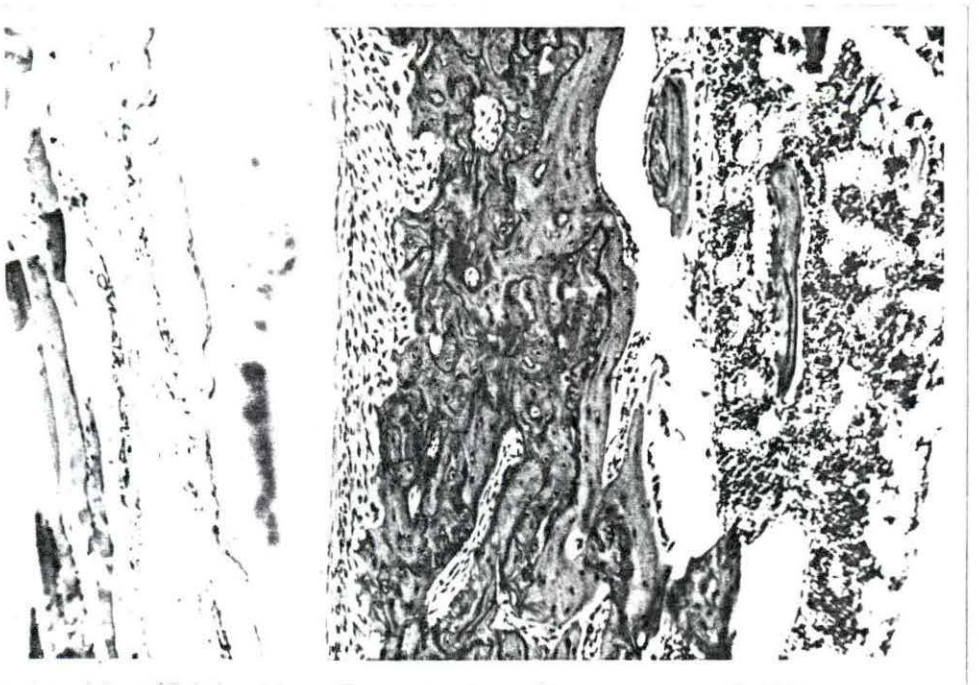


Figure 83. Rat (N-33), age, 55 days: A normal thick cortex in a young rat fed the commercial diet for rats. Compare this figure with Rat (A-47), Figure 84. x 166.

Figure 84. Rat (A-47), age, 69 days: A thin cortex of a rat with brachychondroplasia resulting from a severe deficiency in A vitamins. Compare this figure with Rat (N-33), Figure 83. x 166.



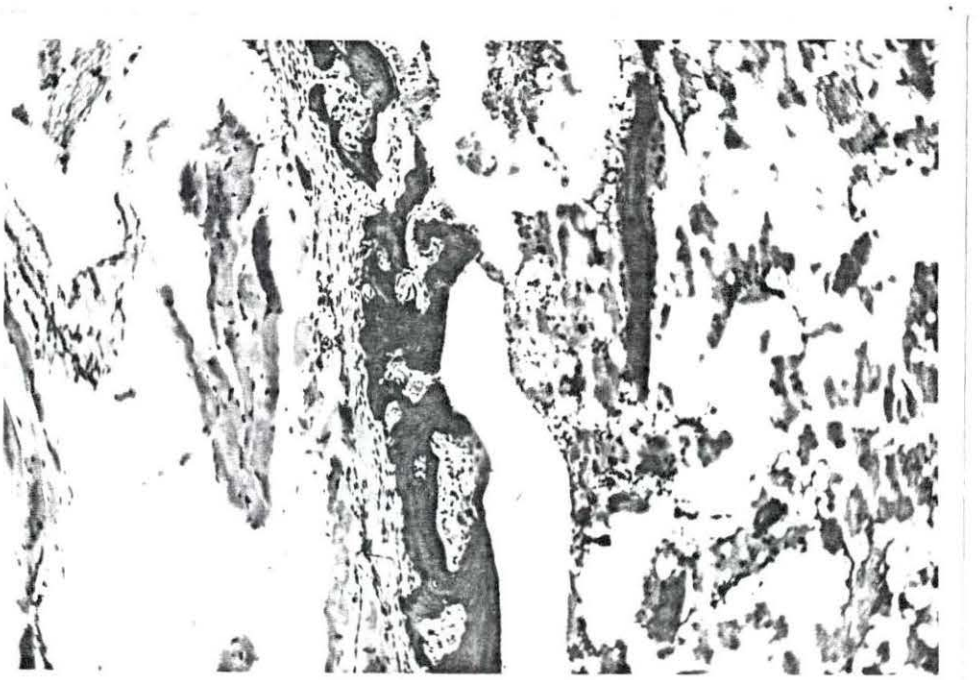
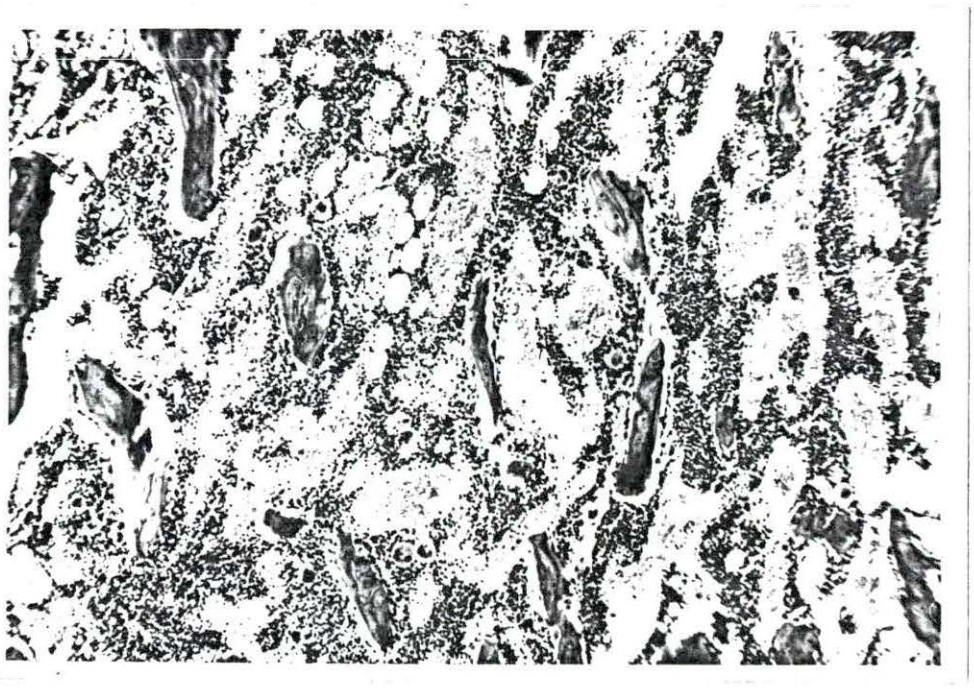


Figure 85. Rat (N-33), age, 55 days: The appearance of normal actively growing bone marrow. Note the cellular appearance of the bone marrow, the actively congested blood vessels and the presence of fat cells. Compare this figure with Rat (A-47), Figure 86. x 166.

Figure 86. Rat (A-47), age, 69 days: Severe suppression of the growth of the bone marrow of a rat deficient in A vitamins. Note the absence of the cells of the bone marrow, the passively congested blood vessels and the numerous fat cells. Compare this figure with Rat (N-33), Figure 85. x 166.



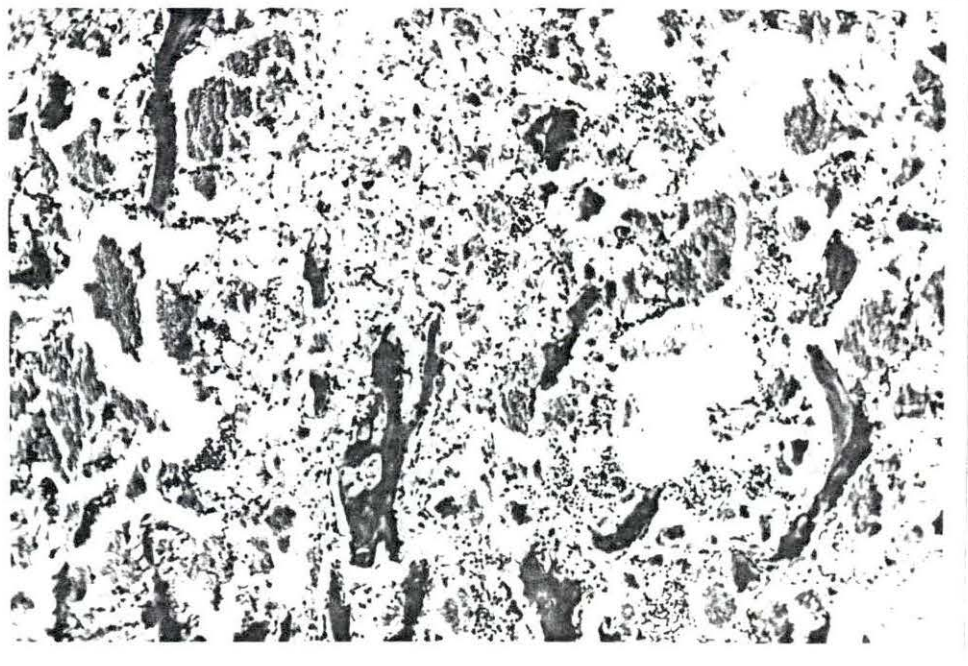
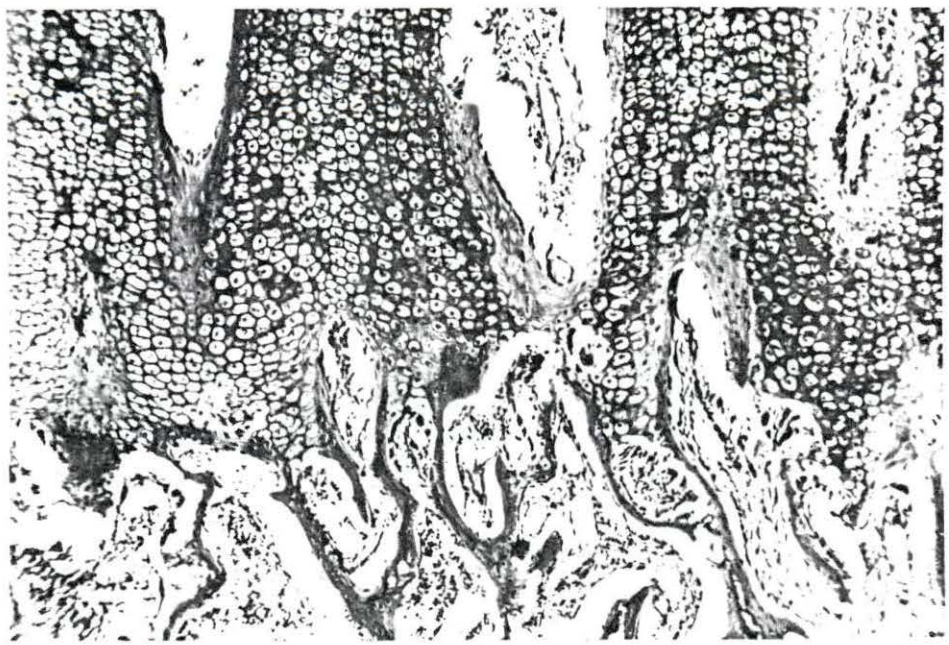


Figure 87. Chick (R-27), age, 28 days: Vesicular cartilage near the epiphyseal-metaphyseal junction of a chick with severe experimental rickets. This chick was fed Rachitogenic Diet No. 2. Note the thick layers of osteoid tissue deposited on the surface of the vascular channels. Compare this figure with Chick (M-19), Figure 88. x 166.

Figure 88. Chick (M-19), age, 20 days: Vesicular cartilage near the epiphyseal-metaphyseal junction of a chick with moderate experimental rickets. This chick was fed Ration A. Note the layers of osteoid tissue bordering the vascular channels and the dense reticular connective tissue in the intertrabecular spaces. Compare this figure with Chick (R-27), Figure 87. x 166.



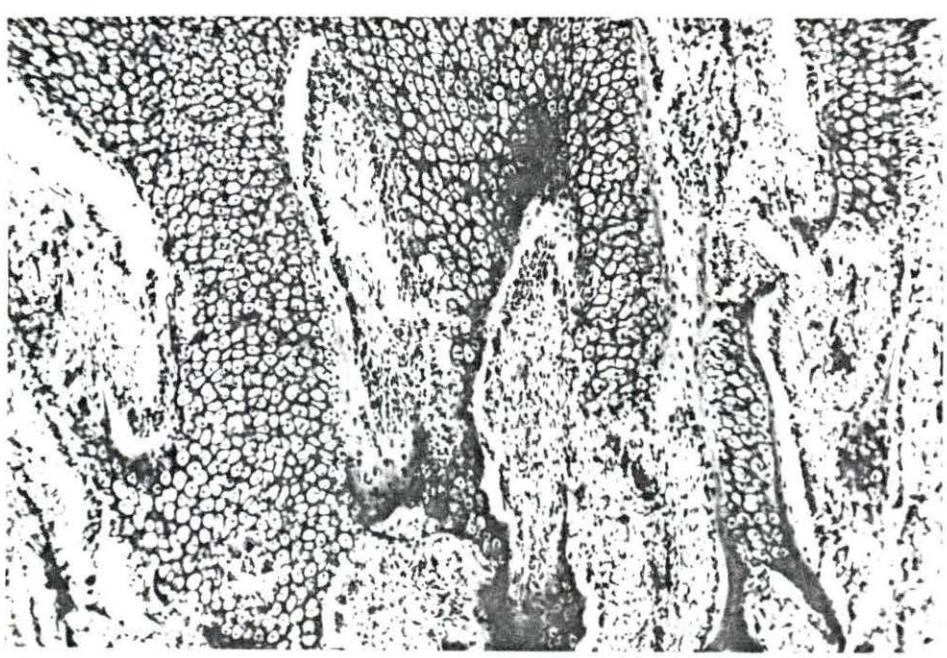
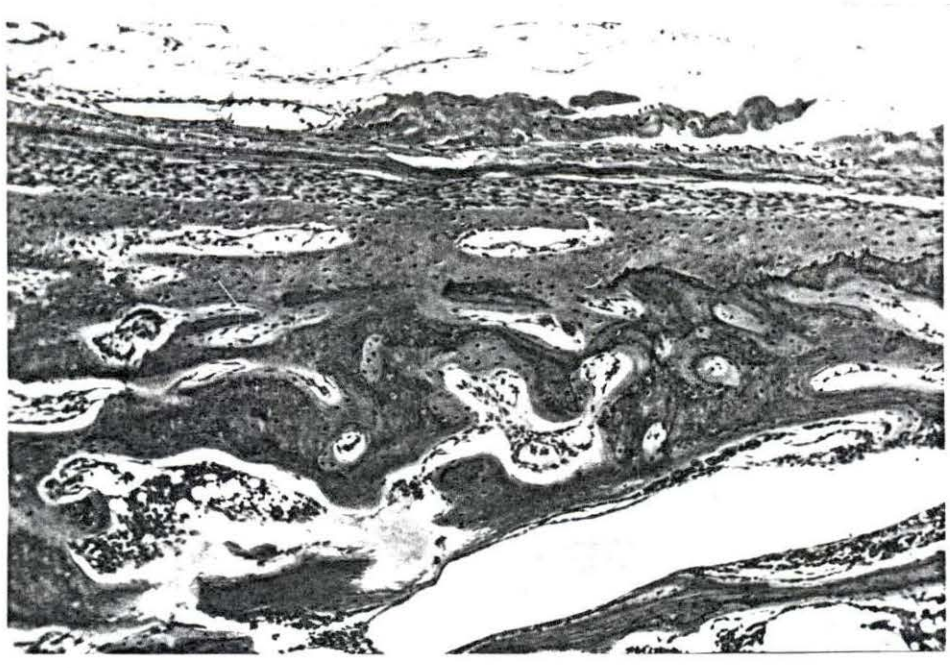


Figure 89. Chick (R-57), age, 58 days: The cortex of a chick with severe experimental rickets. Note the thick layers of osteoid tissue on the periosteal surface of the cortex. x 166.

Figure 90. Chick (R-57), age, 58 days: The epiphyseal-metaphyseal junction of a chick with severe experimental rickets. Note the thin layers of osteoid tissue on the surface of slightly mineralized bone and the necrotic vesicular cartilage undergoing metaplasia to pseudo-osteoid tissue. x 166.



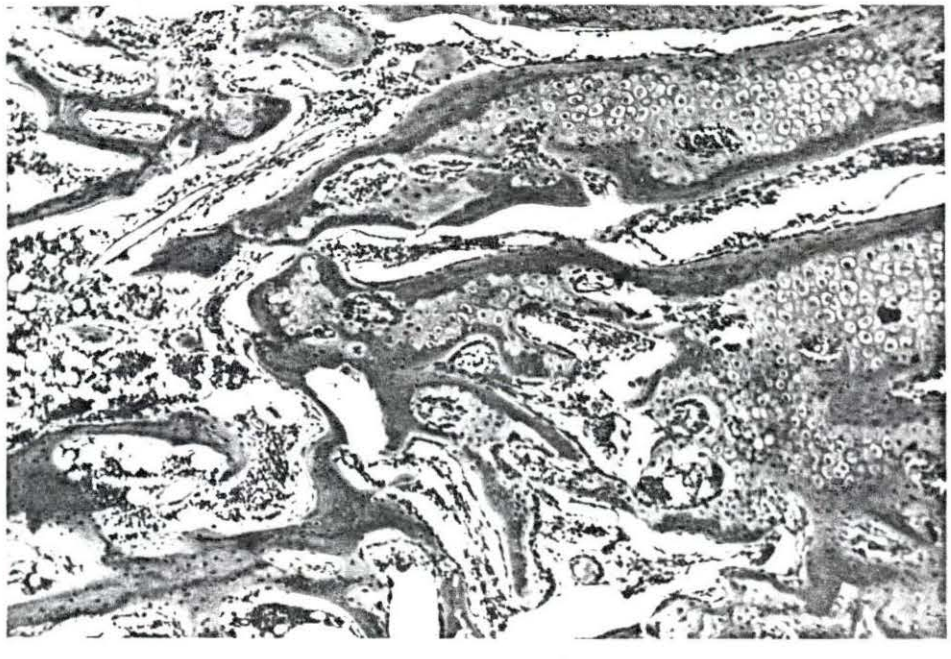
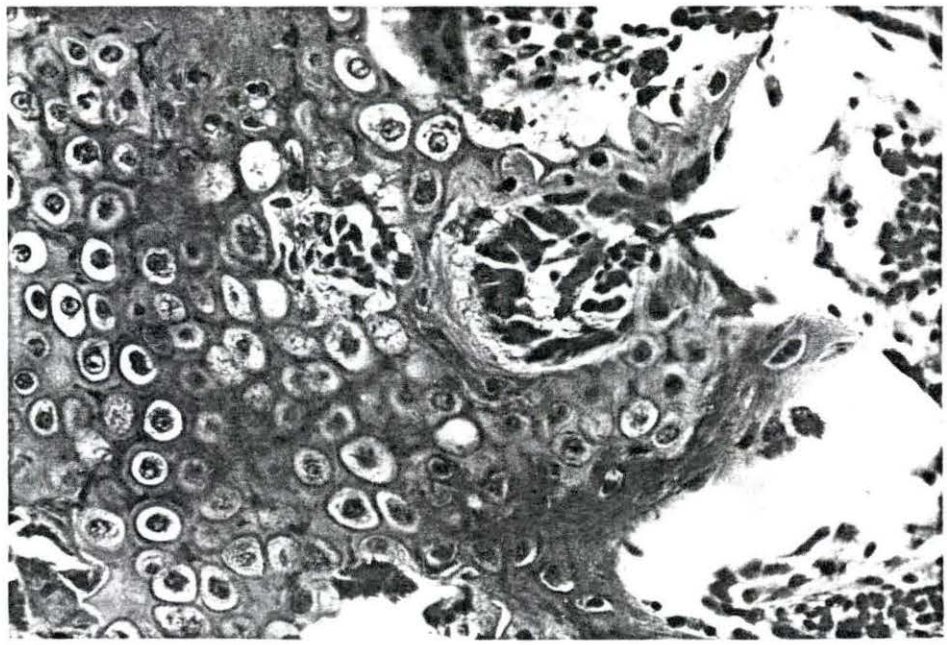


Figure 91. Chick (R-57), age, 58 days: Active cuboidal osteoblasts in the metaphysis of a chick with severe experimental rickets. 714 x.

Figure 92. Chick (M-19), age, 20 days: Active cuboidal osteoblasts in the metaphysis of a chick with moderate experimental rickets. Note the mineralized bone, the thick layer of osteoid tissue and the large number of fibroblasts in the dense reticular connective tissue in the intertrabecular spaces. x 714.



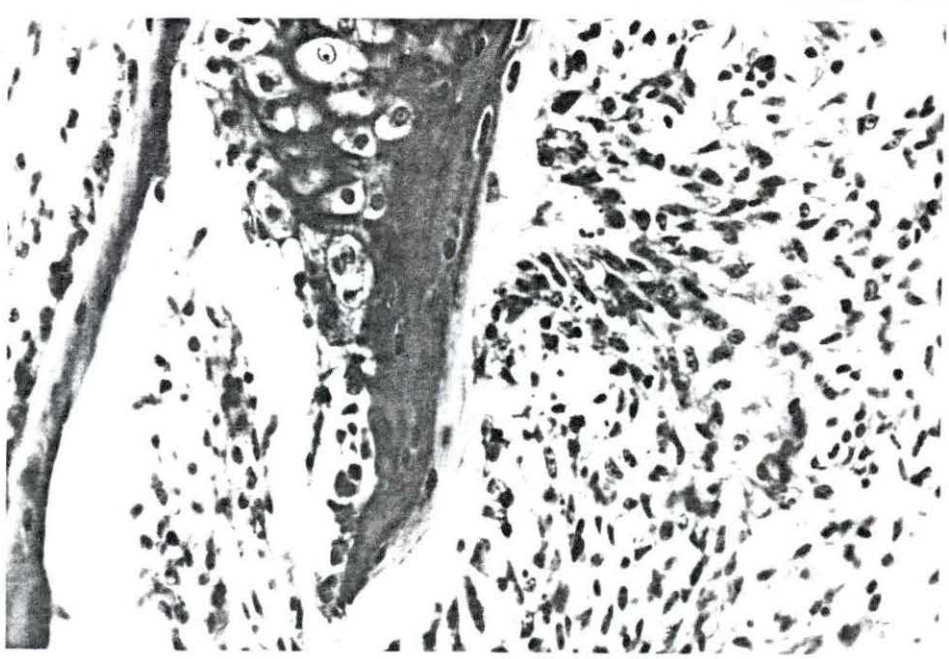


Figure 93. Chick (M-19), age, 20 days: The periosteum of a chick with moderate experimental rickets. Note the absence of osteoblasts and osteoid tissue. Note also the presence of numerous large multinucleate syncytial cells and the active fibroblasts. x 714.

Figure 94. Chick (M-27), age, 28 days: Trabeculae in the metaphysis of a chick with moderate experimental rickets. Note the numerous large multinucleate syncytial cells and the active osteoblasts. x 714.