PATHOGENESIS OF OSTEOPETROSIS IN CHICKENS

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ABSTRACT

PATHOGENESIS OF OSTEOPETROSIS IN CHICKENS

By Vance L. Sanger

The experiment was designed to determine the earliest date at which lesions of experimentally produced osteopetrosis appear and to follow the progressive development of the lesions grossly and microscopically. One hundred, day-old, Regional Poultry Laboratory (RPL), line 15 I chicks were inoculated intraperitoneally with 0.2 ml. of RPL strain 12, preparation L 29, lymphomatosis virus. Twelve control chickens of the same strain and hatch were used. All chickens were identified by wing band number. The numbers were randomized and a group of experimental birds and their corresponding controls were killed each week starting on day 43 after inoculation. The decision for killing the first group on day 43 was based on preliminary work which indicated that this was about as early as lesions could be expected.

Laboratory procedures included radiographs of the long bones of the legs, hematocrits, hemoglobin levels, differential leucocyte counts, serum alkaline phosphatase measurements and gross and microscopic descriptions of lesions in both soft and hard tissue.

Well-developed gross lesions were present on day 43. The lesions nearly always appeared first at the center of the tibial diaphysis at the junction of the posterior edge of the fibula and the tibia. From

here the lesion spread around the circumference of the bone as well as longitudinally, giving the bones a fusiform appearance. The ends were never affected.

Microscopically, the lesions appeared to develop subperiosteally first and appeared at the endosteum only after the subperiosteal lesion was well developed. Or else if the subperiosteal and endosteal lesions started simultaneously the subperiosteal lesion developed at a more rapid rate. The osteoblastic layer of the periosteum was hyperplastic, sometimes measuring 20 to 30 cells in thickness. Spongy bone was formed at a rapid rate. Haversian systems did not form. Instead, the areas that simulated Haversian canals remained much larger than normal and developed into irregular, large spaces, sometimes joining others nearby. These large spaces contained fibrous tissue, one or more capillaries, and were lined by a row of osteoblasts. Bony spicules developed which reached from the surface of the original, normal, cortical bone to the periosteum. Both the spicules and spaces were reoriented from a plane parallel with the longitudinal axis of the long bone to a perpendicular plane. The lesions grew simultaneously in depth on the bone surface, in length along the shaft and in circumference around the shaft.

By a means which could not be determined, a subperiosteal lesion gradually penetrated the original, cortical bone and reached the endosteum and from this point, spongy bone spicules developed in the marrow cavity, gradually reducing its lumen. Or else the lesion started at the point of deepest development at the time the periosteum and endosteum were in close apposition. It then grew both endosteally and subperiosteally at the same time from that point rather than that

the lesion penetrated and replaced original, normal, cortical bone.

Osteoclastic activity was not part of the lesion. The lesion was progressive and proliferative in nature and not degenerative. The characteristics of malignancy were not evident in the osseous lesion. No evidence of invasion of nearby tissue nor metastasis to soft tissue was encountered.

Early lesions were visible grossly and microscopically before they were visible on radiographs. The bones from some chickens with osteopetrosis were easily broken.

Soft tissue lesions included a proliferation of the intima of some arteries and veins to the point the lumens were partially or completely closed. Early bile duct and pancreatic carcinomas were present, and were probably a result of the action of the lymphomatosis virus. Some spleens contained amyloid deposits. Focal necrosis was present in some livers. This was attributed to a contaminant virus described by other workers that was carried in the inoculum.

Hematological studies were limited in significance. Serum alkaline phosphatase measurements were inconclusive.

PATHOGENESIS OF OSTEOPETROSIS IN CHICKENS

By

Vance LaVerne Sanger

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I. INTRODUCTION

Avian osteopetrosis is a disease which usually affects the long bones but occasionally may affect every bone in the body. Initially, in a long bone, a focal lesion appears on the surface of the midshaft of the diaphysis and gradually grows both longitudinally and in circumference until the entire bone may be affected with the exception of the articular surfaces.

The lesions usually become clinically visible in the second or third month of life. In uncomplicated cases, the long bones become markedly enlarged with roughened, irregular, convex surfaces. Gaits are affected and the chickens become anemic. In less severe cases, the lesions may escape notice until the birds are dressed for market. Generally males are affected more often than females.

Osteopetrosis is included in the leukosis complex because it can be reproduced by the leukosis virus and many times it is accompanied by some other form of the disease. Two other forms of leukosis which frequently accompany osteopetrosis are lymphomatosis and erythroblastosis. When either of these is present, it, rather than osteopetrosis, is usually responsible for the death of the bird.

Osteopetrosis can be experimentally transmitted and numerous reports of such experiments have been published. No report, however, has shown the earliest age at which lesions appear in chicks inoculated at 1 day of age with the virus and no report has described the progressive development of lesions from the earliest moment of change to well-developed bone changes. It seemed desirable to attempt to answer

these 2 points in question which would contribute to the knowledge of the disease. Thus the objectives of this study were: (1) to determine the earliest date at which lesions were present either on radiographs of the long bones of the legs, by gross examination or by microscopic study of representative sections of several bones, and (2) to describe both the gross and microscopic development of the lesions up to a well-developed definitive point.

II. REVIEW OF LITERATURE

A. Osteoperiositis

Besnoit and Robin (1922) described a condition in a year-old male chicken and published gross photographs of the long bones which had the typical appearance of osteopetrosis. The chicken was thin and emaciated and walked haltingly and with difficulty. He was negative to the subcutaneous and intradermal tuberculin skin tests. At necropsy the viscera and joints were negative. The muscles were atrophied and the diaphyses of the long bones were hypertrophic and fusiform in shape. The diameter of the affected bones was about 3 times greater than normal. On cross section the medullary canal was reduced in size. The bony wall was thickened and the bone had a homogeneous appearance.

Microscopically the Haversian canals were enlarged; the lamellae were small and completely disorganized; the cells presumably osteocytes were round or elongated and connective tissue was present. New blood vessels were forming.

They stated that the condition appeared specifically as inflammation and diagnosed the bone lesion as osteoperiostitis. They implanted a small piece of bone subcutaneously in a rabbit and after 3 months recovered tubercle bacilli from the rabbit. They concluded that this was primary tuberculosis of the bone, but stated that the lesions were not typical for those of tuberculosis.

Bell and Auger (1924) reported a condition in a chicken which probably today would be called osteopetrosis. The long bones of the legs and wings were enlarged and spindle-shaped. Microscopically, the periosteum was thickened and the osteogenic layer was hyperplastic. Beneath

this was a layer of newly formed bone which had been deposited on the original, normal, compact bone. This same kind of newly formed, spongy bone partially filled the medullary canal. There were no visceral lesions. They raised the question as to whether this was a chronic avisceral form of tuberculosis with only scattered tubercle bacilli present without the formation of liquified or caseous pus. They concluded that the condition they described was primary in the bones, and therefore was apneumonic and avisceral and not related to tuberculosis.

Pugh (1927) described a condition which he called osteoperiositis that probably was osteopetrosis. He described the disease in 7 of 44 affected chickens, all from the same flock. The disease appeared in the sixth to seventh month of life and was not limited to 1 breed. Mostly males were affected. There was enlargement of the long bones of the legs and wings with thickening in the middle of the diaphyses and obliteration of the medullary cavity. The new bone was softer than normal bone, yellow in color and cut easily with a knife. The lesions gradually progressed toward the bone ends but the ends were not enlarged. The total weight of the bone was increased. The periosteum was thickened. Microscopically, the process was one of osteitis and periosititis with laying down of new osseous tissue. There were no other signs of ill health.

Carpentier (1931) reported a single case of a condition which was probably osteopetrosis. A male chicken, 3 months of age, was noticed to be lame and less well developed than others of the same age. He was killed at 5 months of age; his weight was 1400 Gm. Upon examination the long bones of the legs and wings were enlarged and spindle-shaped

with the greatest enlargement in the centers of the diaphyses tapering to the ends. The ends were unaffected. The subperiosteal regions were affected with the new growth of osseous tissue which was harder than normal. The bones were also heavier than normal bones from chickens of the same age. The diseased tibia weighed 39 Gm. compared to 12 Gm. for a normal bone. The medullary canal was normal. There were no visceral lesions.

He originally thought this was tuberculosis of the bone but tuberculosis was eliminated after studying the flock history and killing the 100 remaining chickens in the flock for examination.

Bayon (1934) described 2 unrelated cases of a bone disease in chickens.

"One, a cross-bred cockerel, had thick bones of the legs. Microscopic examination of the bones revealed a condition analogous to Paget's disease in man and which in fowls has been previously described by Pugh (1927). The inner layers of the internal surfaces of the bones are eroded and carried away and the bony substance so obtained is deposited on the outer surface of the skeleton, thus rendering the bones abnormally thick and deformed. This perverted destruction and reconstruction are clearly evident in the bones of another case."

He did not know the cause. The appearance of the affected metatarsal bones in the photograph was indistinguishable from the condition recognized today as osteopetrosis.

Patay (1935) reported on several bone malformations 1 of which may have been osteopetrosis. Several hens developed a progressive thickening of the long bones of the legs and wings and sternum. Walking was difficult and affected birds remained small. Cross sections of the long bones revealed a complete absence of the medullary canal. Microscopically, there was evidence of both bone formation and bone destruction. The medullary canal was filled with bone spicules between which were canals

and spaces. Osteoblasts, multinucleated giant cells and fibrous tissue filled the spaces. On the surface of the bones, osteogenesis was taking place and the surface had a roughened appearance. Parathyroids were hypertrophic. There were no other lesions.

Brocket (1935, a) found a hypertrophic osteopathy in a bird which had been killed and inspected for market. All long bones were affected but not the skull or vertebrae or pelvis. The bones were large in the center and tapered toward the ends. The tibias were most seriously affected. The new bone appeared spongy. The medullary cavity seemed slightly larger when compared to a normal bone but the bone size was due to an increase in bone substance. The new bone was hard and difficult to saw. The bony surface was abnormal and had transverse lines. The viscera and skin were normal.

He reasoned that this condition started with an acute general infection, probably of respiratory origin, and spread to the bones, with a possible endocrine predisposition which resulted in hyperossification. He listed 7 possible diagnoses: pneumo-hypertrophic osteitis, acromegaly, Paget's disease, tuberculosis, gigantism, osteosarcoma and vitamin deficiency. In a later communication he concluded this was the same condition described by Pugh (Brocket, 1935, a).

B. Osteomyelosclerosis

Seifried and Sassenhoff (1940) described osteomyelosclerosis in chickens. This was a disease of the spongiosa and not the compacta. The spongy bone eventually filled the marrow spaces but the cortex remained unchanged. Bones of the body skeleton were affected but the bones of the extremities remained unchanged. This condition was not like osteopetrosis as it appears today.

Seifried (1941) described a disease of the bones and of the blood forming organs of chickens which did not resemble any other disease in domestic animals including the marble-bone disease described by Jungherr and Landauer. However, it did appear to be similar to the group of diseases in humans known as osteomyelosclerotic blood diseases.

Szücs (1952) described a condition which he called osteomyelosclerosis. This disease was different from osteopetrosis but some features were similar. The spongy substance of the epiphyses and marrow cavities gradually became more compact. In advanced cases the entire marrow cavity was filled with bone substance. There was no evidence of leukosis in the experimental birds and the author did not mention any roughening of the periosteal surfaces of the affected bones. The disease appeared at about 1 year of age; the cause was not determined.

Theiss (1944) described a condition in 62 chickens which he called "osteomyelosklerose". The number of affected chickens increased as they grew older. He did not describe the condition except to quote other authors. A gross photograph of the bone reveals a condition indistinguishable from what is known today as osteopetrosis.

C. Osteitis Deformans

Venkataraman (1936) reported that a hen and a cock developed diffuse thickening of long bones and anemia and emaciation. The medullary cavity of long bones contained dense compact bone resembling ivory. No cause was found. He called the condition osteitis deformans.

D. Osteopetrosis

Karshner (1926) was the first to use the term "osteopetrosis" for a human disease called "marble bone" or Albers-Schoenberg disease. In this hereditary disease he found an increase in thickness and density of the cortical portion of the osseous system. He felt that "osteopetrosis" suggested fragility as well as hardness, limestone rather than marble.

Jungherr and Landauer (1938) were the first to transmit the disease experimentally. In the early stages, the metatarsi developed an anterior convexity and thickening and irregularity of the surfaces. Articular surfaces were unaffected. Affected bones were more resistant to fracture than normal bones.

Microscopically, in the early stages, medullary fibrosis, increased osteoclasia and degeneration of old bone were found. Trabeculae showed stippling with a fine basophilic dust-like material.

Newly formed bone consisted of many irregular-sized blood spaces which contained a dense, central, fibrous material, osteoblasts and a few osteoclasts. There was a reduction in the marrow cavity in affected bones. Later, Jungherr (1959) stated that in comparison with other forms of leukosis, the occurrence of osteopetrosis was rare. In the initial phase of the disease he found sequestration, granular degeneration of old trabeculae, marrow fibrosis and increased osteoclasia. These changes were accompanied by new large-celled, vascular, fibrous bone tissue. Later, new bone gradually replaced the original spongiosa and compacta while osteoclastic activity regressed. In the arrested phase, new bone lamellae appeared condensed, hypercalcified and subdivided by numerous,

thick, irregular, cement lines. Secondary anemia was common because of the progressive reduction of hemopoietic tissue.

Brandly (1941) injected embryonated eggs with whole blood and washed erythrocytes. In 7 to 10 days, the liver, spleen and diaphyses of the long bones of the legs and wings were enlarged. Microscopically, bone changes were similar to those seen in osteopetrosis. Brandly, Nelson and Cottral (1941) produced lymphomatosis and osteopetrosis with strain 3 of their agent. Osteopetrosis occurred in 9 percent of inoculated birds. Some of these lesions were visible grossly. Others were found microscopically. More males than females were affected. Brandly, Nelson and Cottral (1942) obtained their strain 3 inoculum from White Leghorn hens suffering from ocular lymphomatosis. Strain 3 inoculum caused visceral, neural and ocular lymphomatosis and osteopetrosis. One chicken inoculated at 21 days of age had clinical osteopetrosis 57 days later. Another chicken developed osteopetrosis 300 days after inoculation. Osteopetrosis was found in 9 percent of injected birds. The sex ratio of 25 affected birds was 17 males and 8 females. There was hyperpyrexia of affected bones as well as a marked enlargement in the diameter of the long bones. A few skeletal bones were affected. Microscopically, there was increased cellular activity of the periosteal region, blood spaces were enlarged and irregular; leukemic blood changes were not found in the osteopetrotic birds although they frequently were anemic. The medullary cavity was obliterated in some bones. Gross examination of the parathyroid glands in osteopetrotic birds revealed no changes.

Duran-Reynals (1942) injected chickens with Rous sarcoma virus and produced osteopetrosis. The gross and microscopic appearance of the lesions was the same as that described by Jungherr and Landauer. The parathyroid glands were not enlarged. Osteopetrosis did not develop in ducks injected with Rous sarcoma virus. Bieley (1943) found osteopetrosis in a chicken. The long bones were enlarged and irregular in outline. The vertebrae and skull were unaffected and there were no soft tissue lesions. Moynihan (1943) reported osteopetrosis in 7 chickens, 1 male and 6 females. The chickens were anemic and oligocythemia was a constant feature. Leukemia was not observed. All bones except the skull and vertebrae were affected. Lesions were characterized by hypercalcification resulting in partial or complete obliteration of the marrow cavity. Bone surfaces were rough and irregular and diseased bones were several grams heavier than bones from healthy chickens of the same age.

Thiersch (1944) inoculated blood from human patients suffering from myeloid leukemia into 11-day-old chick embryos. Only a small percentage of the injected chicks survived; of 12 chickens hatched from the inoculated embryos, 5 developed osteopetrosis. Similar lesions were obtained by injecting day-old chicks. Osteopetrosis could be induced with blood from 3 different patients with chronic myeloid leukemia. Injection of blood from human subjects with chronic lymphoid leukemia did not induce osteopetrosis.

Coles and Bronkhorst (1946) observed osteopetrosis occurring equally in both sexes. Affected bones were exceptionally hard and did not fracture easily. The ends of the bones were unaffected. They

reported that susceptibility to osteopetrosis was an inherited recessive character.

Burmester, Prickett and Belding (1946) inoculated 2-3-day-old-chicks with cell-free preparations of RPL 12 lymphoid tumor. By 10 weeks of age, clinical manifestations of osteopetrosis were seen and by 6 months of age, 41 percent had osteopetrosis.

Affected bones were irregular in outline, warmer than usual and hard to the touch. The pathological changes were confined to the diaphysis. The marrow cavity was reduced in diameter by deposition of spongy bone. The periosteum was hypertrophic. Microscopically there was hyperplasia of the periosteum and an increase in its thickness with formation of new and abnormal cancellous bone beneath the periosteum. Numerous irregularly placed marrow cavities contained hyperplastic tissue which appeared to be bone marrow. The normal diaphyseal architecture of bone was completely altered.

Burmester and Cottral (1947) stated that there was some evidence to suggest that osteopetrosis and visceral lymphomatosis were produced by different agents and that either might remain latent in recipients and become overt in subsequent passages.

Burmester (1947) found that RPL strains 18 and 21 produced osteopetrosis as well as visceral tumors.

In summarizing the work at the Regional Poultry Research Laboratory, Winton (1951) reported that visceral lymphomatosis and osteopetrosis had been transmitted with cell-free filtrates which indicated that these forms of leukosis were caused by a filterable virus-like agent or agents. He suggested that osteopetrosis may have a causative agent separate from the one causing visceral lymphomatosis.

Pearce and Brown (1948) and Pearce (1948, 1950a, 1950b) described osteopetrosis in an inbred group of rabbits. The condition was an inherited, lethal characteristic that somewhat resembled Albers-Shoenberg disease in man. Bone lesions, as well as several other anomalies, were present at birth. The bones were hard but brittle. Spontaneous fractures were rare.

Radiographs revealed clubbing of the ends of ribs and long bones, shortening of the long bones, increased bone density, reduction of the marrow cavity and scanty marrow.

The periosteal surfaces of the bones appeared normal but there was size reduction. The bones felt tough and fibrous when cut with a knife.

Osteocytes were numerous, many were pyknotic and a variation in size and staining reaction was noticed. Groups of 2 to 6 osteocytes were crowded together and formed small cellular foci. When compared to normal bone, osteoclasts were reduced in number in some areas and more numerous in other fields.

Fibrous tissue was prominent in the marrow cavity among the trabeculae and extended into the cortex and reached the periosteum in some places. Small cysts were frequently seen in the fibrous tissue. There was actual failure of development of marrow cavity and marrow tissue.

The primary parathyroid glands were enlarged and aberrant glands were found along the cervical area. This was in contrast to small glands and only a few aberrant glands in controls. Microscopically more cysts were found in the glands of the affected rabbits than in those of controls.

Hemopoietic centers were far more numerous in the livers of affected rabbits than in those of the controls.

Davakula (1953) reported that "in comparison with the frequency of the occurrence of other forms of the avian leukosis complex, osteopetrotic lymphomatosis was rare." "Clinically, observation and palpation of long bones, as the matatarsi, may reveal abnormal convexities or irregular thickenings of the affected regions. Gross alterations of the long bones were observed in various degrees depending on the stage of development of the disease. Hematological and radiological examination may also confirm the evidence of avian leukosis complex."

In a discussion of the classification of the different pathological expressions of leukosis, Campbell (1954) suggested that osteopetrosis should be removed from the leukosis complex and set apart as a distinct pathological entity.

Over a 2-year period in the examination of 4459 chickens for diagnostic purposes, Jordan (1956) found 2 birds with osteopetrosis.

Holmes (1958a, 1958d, 1959a, 1959e, 1961c, 1961f, 1963g) published a series of papers in which he described the results of his experimental work on osteopetrosis in chickens and turkeys and observations he made as a result of the work. He transmitted osteopetrosis by the use of whole blood, unfiltered and filtered plasma and bone marrow. The earliest lesion was detected by palpation at 35 days of age. Lesions developed later in turkeys than in chickens. The earliest radiological detection of osteopetrotic lesions was at 37 days following inoculation at 1 day of age. Embryos which were inoculated and allowed to hatch developed osteopetrosis. Lesions were characterized initially by subperiosteal hyperemia followed by the formation of new subperiosteal bone. The earliest changes occurred on the diaphyses of the long bones. The entire skeleton was sometimes affected, including the skull and vertebrae. Bones grew wider and denser than normal. The subperiosteal lesions

were later followed by endosteal lesions with narrowing and obliteration of the marrow cavity. Initially the radiological appearance of very early lesions was that of periostitis. This periosteal reaction was the characteristic response of long-bone periosteum to any stimulus and may appear in a number of conditions. These primary changes in osteopetrotic lesions were constructive and not destructive and therefore osteopetrosis should not be considered a neoplastic disease. He further suggested that osteopetrosis may be caused by a virus separate from the leukosis virus; therefore this disease should be considered as a separate entity from the leukosis complex and avian neoplasia. Cortisone injections did not influence the development of the disease in young chicks. Osteopetrosis resembled infantile, cortical hyperostosis (Caffey's syndrome).

In testing strains of leukosis virus used for research which were obtained from places other than the U. S. Regional Poultry Research Laboratory, Burmester, Walter, Gross and Fontes (1959) found that cell-free preparations of myeloblastosis strain A caused osteopetrosis but similar preparations of erythroblastosis strain R did not. Burmester, Gross, Walter and Fontes (1959) also found that osteopetrosis was common in 5 of the 7 preparations of RPL 12 virus given parenterally which they tested. It did not occur in chickens inoculated with oral washings, fecal extracts or embryo extracts of naturally infected hens.

Gross, Burmester and Walter (1959) reported that in the early stages of uncomplicated osteopetrosis, diseased birds had an anemia, unthrifty appearance.

Many suffered from leg fractures and all long bones could be crushed easily at necropsy.

Microscopically the first visible alteration was an increase in size and number of the cells in the deep layer of the periosteum, endosteum and Haversian canals. This proliferation progressed rapidly, and assumed neoplastic features as evident by the partial lysis of the compact bone. New cartilaginous tissue was laid down in the lytic spaces and mineralization followed. This produced bone with an imperfect lamellar system.

A markedly atrophic and fibrous spleen frequently accompanied osteopetrosis and occasionally a cirrhotic liver. Osteopetrosis was considered a neoplastic disease. However, a question regarding malignancy was raised because the proliferative process eventually subsided.

Ressang (1960), in a discussion of the avian leukosis complex, reported that leukosis had been recognized in Indonesia since 1928 but was not considered to be of economic importance. The visceral, ocular and neural forms had been diagnosed but osteopetrosis, erythroblastosis, granuloblastosis and myelocytomatosis had not been diagnosed.

Darcel (1960) stated that osteopetrosis was a non-neoplastic bone malformation.

Campbell (1961) found that inoculation of day-old chicks with blood from a florid case of osteopetrosis produced well-developed lesions in 12-14 weeks, especially in males. Affected bones had a thickened periosteum and were highly vascular.

The initial lesion on X-ray was an endosteal bone proliferation followed by progressive laminar deposition of periosteal bone. The medullary cavity was eventually obliterated by new bone with an abnormal architecture due to enormously enlarged and elongated Haversian canals

extending centripetally toward the shaft center. These vascular spaces contained no marrow but had a scanty fibro-cellular tissue. Anemia followed which stimulated an extramedullary myelopoiesis in the liver and kidneys.

He further stated that osteopetrosis was not a neoplastic condition but rather an osteopathy characterized by hyperplasia. It was associated with a virus. It had not occurred in conjunction with lymphoid and myeloid leukosis and spontaneous development was unknown. No experimentally induced osteopetrosis cases developed any leukosis. It cannot be said that the leukoses and osteopetrosis had a common etiology.

DeVolt (1961) reported that osteopetrosis was being encountered with increasing frequency in turkeys.

E. Alkaline-Phosphatase

Changus (1957) analyzed osteoblasts for alkaline-phosphatase activity in both malignant and nonmalignant diseases. He found that in all bone lesions from patients with fibrous dysplasia of bone there was high alkaline-phosphatase activity in the osteoblasts along the margins of bone trabeculae and in almost all those cells distant to the bone trabeculae that have been called fibroblasts.

The periosteum removed from tibias not directly involved with specific disease had abundant alkaline-phosphatase activity in the inner layer adjacent to the bone. Histochemically, these cells are interpreted as osteoblasts. The outer layer of the periosteum had little or no alkaline-phosphatase activity, and the cells composing this layer were interpreted as histochemical fibroblasts.

In 5 cases of fibrous dysplasia of bone the serum alkaline-phosphatase levels were within normal range and varied from 2.5 to 3.7 Bodansky units. The inner layer of the periosteum was rich in alkaline-phsophatase activity but the outer layer was poor in or devoid of this activity.

Osteogenic sarcoma of the bone was high in histochemical alkaline-phosphatase activity while fibrosarcomas of the bone were devoid of this activity.

The data presented here demonstrated that the osteoclasts, giant cells and macrophages all had acid-phosphatase activity and no alkaline-phosphatase activity. This indicated that the osteoclasts, like other reactive giant cells, were derived from epitheloid cells or macrophages and not from pre-existing osteoblasts or fibroblasts. The findings also indicated that alkaline-phosphatase does not participate in decalcification of the bone but that acid-phosphatase does.

Fibrous dysplasia is an exaggerated hyperplasia or response of osteoblasts to some unknown stimulus.

Bell et al. (1959) found an increase in plasma alkaline-phosphatase in laying hens suffering from cage layer fatigue. The bones were extensively decalcified and flexible. Histologically, there was marked osteoclastic activity and thinning of the compact and spongy bone.

Siller (1959) described an osteogenic sarcoma from the proximal end of the right tibia of a 7 year old hen. Mitotic figures were present and nucleoli of the osteoblasts were large and prominent in the primary tumor. There was invasion of muscle and metastasis to the kidney and lungs. The metastatic tumors resembled the primary tumor and were

unencapsulated and infiltrative. Calcification was present and mitotic figures were numerous. The plasma alkaline-phosphatase measured 360 units compared to 25 to 120 units for a normal non-laying hen.

Bell (1960) found that the plasmas of immature birds (5 to 6 weeks old) of both sexes had alkaline-phosphatase levels up to 10 times those found in non-laying adults. In the hen, the average level of plasma enzymic activity increased by about 50 percent when laying began. At this time, also, wide fluctuations became apparent. He attributed the rise to increased osteoblastic activity.

Hurwitz and Griminger (1961) found enlarged parathyroids, elevated plasma alkaline-phosphatase and loss of bone material in calcium-deficient hens.

F. GAL Virus

Sharpless and Jungherr (1961) reported that a Gallus adeno-like (GAL) virus was isolated from a lymphomatous liver which produced areas of necrosis in the livers of experimentally infected chicks. The presence of this virus is transmissible lymphomatous virus preparations might explain earlier reports of a primary necrotizing agent in transmissible avian lymphomatosis.

In his studies on inflammation, Menkin (1948) reported that a substance called necrosin was released by damaged cells and caused thrombosis of small local lymphatics and cloudy swelling, vacuolation and granularity of cytoplasm of liver cells.

G. Thyroid

Goble (1954) described atrophy and degenerative changes in the thyroid glands in dogs infected with Trypanosoma cruzi. He found that

"grossly the thyroid glands were normal in size, configuration and consistency. Microscopically, however, the follicles were small and contained little or no colloid. The epithelium was uniformly cuboidal and showed no tendency to become columnar or to form proliferative papillae. Many of the follicles contained spherical agranulocytic cells with eccentric nuclei. There was no evidence of acute inflammatory process, fibrosis of either capsule or stroma. The changes were diffuse rather than focal.

He believed these changes were caused by thyrotoxic substances but he did not know the site of origin.

H. Nephroblastoma

Walter, Burmester and Cunningham (1962) reported that the BAI strain A (myeloblastosis) virus produced nephroblastomas. These tumors, in turn, were passed and both cell suspensions and cell-free filtrates produced tumors as well as forms of leukosis including granuloblastosis, lymphomatosis and osteopetrosis.

These avian renal tumors should possibly be considered an additional member of the group of diseases known collectively as the avian leukosis complex.

I. Endothelioma

Furth described endotheliomas in chickens injected with his strain 2 leukosis virus. Tumor size varied from microscopic to 7 cm. They developed in situ through proliferation of preformed endothelium. However, many times the endothelial, mesenchymal or mesothelial origin could not be determined. Necrosis and giant cells usually occurred in the tumors. Many tumors formed cavities and channels in which hemocytoblasts and erythroblasts and erythrocytes were present. These blood cells originated from detached, swollen, endothelial cells. Many tumors were accompanied by hematomas. Cells composing the tumors were sarcoma-like or multinuclear giant cells. Some tumors were invasive.

III. MATERIALS AND METHODS

A. Inoculum

The inoculum was preparation L 29 of the RPL 12 lymphomatosis virus maintained at the U. S. Regional Poultry Research Laboratory. The dosage was 0.2 ml. of a 5-percent extract not further diluted, given intraperitoneally. The extract was prepared from the liver of a chicken suffering from erythroblastosis. Preparation of the virus was begun by homogenizing a 5-percent suspension of liver in Sims' solution in a Waring blender for 20 minutes. This preparation was centrifuged for 30 minutes at 20,000 revolutions per minute under refrigeration. The supernatant was removed and again centrifuged for 30 minutes at the same speed under refrigeration. It was then sealed in vials and stored at -70 Fahrenheit until used.

B. Chicks

The chicks were hatched at the Regional Poultry Research Laboratory from line 15 I Leghorns maintained at this laboratory and were inoculated when they were 8 hours old. Only cockerels were used and each chicken was wing-banded at the time of inoculation. One hundred chickens were given virus and placed in an isolation room in a battery for the first 4 weeks; then they were transferred to a separate room and placed on the floor which was formed by wire stretched over 2 X 4" boards placed on edge. Feed was placed in metal trough feeders. Water dripped continuously from a faucet into an overflow cup. Ventilation was by window from the outside.

Thirty-six control chicks from the same hatch were housed similarly but protected from exposure to the virus or to inoculated chicks.

The numbers were randomized, and 15 experimental and 3 control chickens were killed each week starting at 43 days of age.

C. Laboratory Procedure

Just prior to killing each bird, 2 milliliters (ml.) of blood were drawn from the wing vein or by cardiac puncture. One ml. was deposited in a clean, dry test tube and allowed to clot so that serum could be obtained.

The remainder was placed in a tube containing 0.1 ml. of a 10-percent solution of the anticoagulant, ethylenediamintetraacetic acid (EDTA), which had been oven dried.

Blood smears were made in duplicate, air dried and stained with Wright's stain.

For the hematocrit measurement, a plain capillary tube, 75 millimeters (mm.) long and 1.3 to 1.5 mm. in diameter was filled and one end plugged with a vinyl plastic putty. The tube was placed in a micro-capillary centrifuge and spun for 5 minutes at 11,500 revolutions per minute (RPM). The packed cell volume was read in percent of the total column from a micro-hematocrit reading device.

For hemoglobin determination the acid-hematin method of Bankowski, as described by Fredrickson (1963) was followed. Two ml. of 0.4 percent aqueous ammonium hydroxide was placed in a colorimeter tube. Two-tenths ml. of fresh whole blood measured in a Sahli pipette was added and thoroughly mixed. After 5 minutes, 3 ml. of tenth normal hydrochloric acid was added, mixed and allowed to stand 20 minutes. This solution was then read in a Bausch and Lomb colorimeter which had been standardized

by a hemoglobin sample prepared the same way from dog's blood and compared with the reagent blank and hematin standard.

The clotted blood samples were centrifuged immediately upon returning to the laboratory from the necropsy room where the chickens were bled.

The serum alkaline-phosphatase level was measured on the fresh serum as soon as it could be separated from the coagulated blood sample. This enzyme is not very stable; therefore the test was completed as quickly as possible in order to avoid any loss of enzyme activity. The enzyme was measured in Sigma units according to the method described by the Sigma Company (1960).

Radiographs were made of the legs of 97 of the 112 birds in the experiment. The other 15 either died at an early age or at a time that was not convenient for taking the radiograph. The skin and feathers covering the femurs, tibias and fibulas was removed. The flesh was left intact. The legs of each pair were placed on their lateral surfaces with the femurs directed toward the top of the film so that the left leg facing the viewer in the radiograph was the right leg of the bird.

Kodak, non-screen, ready-pack X-ray film was used. The focal distance was 36 inches and the exposure was 15 milliamperes per second (MAS) in 44-kilivolt peak (KVP).

D. Necropsy

Relatively complete necropsies were done on all chickens. Brain, thyroid, thymus, lung, heart, proventriculus, duodenum, pancreas, liver, spleen, kidney, adrenal, gonad, cranium, and all long bones of the right wings and right legs were saved in 10 percent neutral formalin. Bones of

the left leg and pieces of liver, kidney, spleen, adrenal, duodenum and pancreas were saved in Zenker's fixative. Liver, kidney and adrenal were also fixed in Carnoy's fixative.

E. Histopathologic Techniques

For routine sections all tissues were embedded in paraffin, sectioned at 6 microns and stained with hematoxylin and eosin. Special stains included Masson's trichrome, Van Gieson, Perls' iron stain, periodic acid Schiff (PAS), Hansen-Bock stain for bone, Weil-Weigert's elastic stain, and crystal violet for amyloid. Histologic techniques described by Lillie (1954) and the manual of the Armed Forces Institute of Pathology (1957) were followed.

Bone segments were prepared by sawing cross-sections from the approximate centers of the shafts of long bones and longitudinal sections from the proximal third of the tibia and femur in a line to approximately divide the bone into two equal parts. The longitudinal sections of the proximal ends of the femurs were cut on a medio-lateral plane just anterior to the head. The posterior half was used for sectioning. The tibial sections were sawed on the antero-posterior plane just medial to the fibula. The lateral half was always saved for sectioning and in most instances the stained section included part of the fibula.

A cross section of both frontal bones was taken at the level of the center of the cerebral hemispheres.

The proximal end of the humerus was sawed on a dorso-ventral plane and the anterior half saved for sectioning. Occasionally cross-sections of the radius and ulna were taken from the middle thirds of the diaphyses.

A jeweler's saw with 52 teeth per inch was used for sawing the bone sections.

Bones were demineralized in "Decal*".

*Scientific Products, Evanston, Illinois.

IV. RESULTS

A. Clinical Observations

Chickens suffering from osteopetrosis were stunted in growth and had a pale appearance around the head and wattles and the feathers were rough and ungroomed (Figures 1 and 2). Affected birds walked with a limp or a stilted gait and lifted the feet higher than usual when walking. Some birds sat down most of the time unless disturbed.

Usually the first visible sign of osteopetrosis occurred as a swelling on the metatarsi with gradual enlargement and development of a convex curvature on the anterior surfaces. Palpation of the other long bones of the legs and wings revealed increased size and roughened surfaces. These changes were accompanied by a decrease in the thickness and size of the muscles covering these bones. Affected bones were warmer to the touch than those of normal chickens.

At necropsy, when the lengths of the femurs and tibias of the stunted birds were compared with bones from the same age controls, the bones from the sick birds were shorter than controls but this was considered to be a result of reduced growth rather than the effect of the disease on the individual bones.

B. Gross Pathology

As a result of preliminary work on osteopetrosis at the U. S. Regional Poultry Research Laboratory (Fredrickson and Sanger, 1961) it was decided to kill the first group of chickens at 43 days of age. Fifteen experimental and 3 control birds were killed initially and each

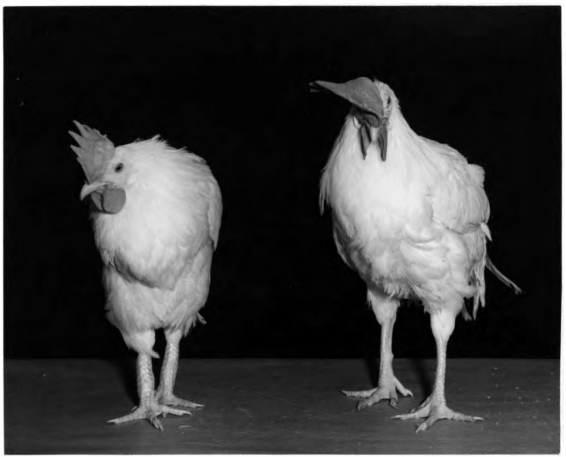

Figure 1. Forty-nine-day-old chickens. Chicken on the left was injected with lymphomatosis virus at 1 day of age and has well-developed osteopetrotic lesions. Compare with figure 2.

Figure 2. Chickens 183 days of age. The chicken on the left was injected with lymphomatosis virus at 1 day of age and has extremely large, osteopetrotic lesions.

week after that until the entire group was gone. Statistically it was found that 3 controls were adequate for each group of 15 experimental birds.

Sixty birds had gross lesions of osteopetrosis. In nearly every instance, the earliest appearance of a gross lesion was on the center of the diaphysis of the tibia at the posterior edge of the fibula. The lesion increased in depth at this point and at the same time advanced rapidly from this point posteriorly around the shaft and toward each end of the bone giving the bone a fusiform appearance (Figure 3). The progress of the lesion anteriorly from the fibula was much less rapid. In advanced lesions the two edges of the lesion eventually met on the anterior surface of the tibia and stopped at the extremities of the shaft so that the ends of the bones were never affected. Occasionally a lesion started first in either the proximal or distal third of the shaft, and on a few bones 2 separate foci were visible at the same time.

On the femur, the first lesion nearly always appeared on the proximal third of the diaphysis on the antero-medial border. From this point it progressed around the circumference of the shaft and also developed distally.

The lesion appeared as a distinct, pale, yellowish focus in contrast to the grayish-white to translucent appearance of the normal bone. Except in the very earliest lesion, it was always elevated above the level of the adjacent bone surface. The periosteum was thickened and the surface of the lesion was less firm to the touch than the normal bone. A knife easily cut through the new osseous tissue until the original compact bone was encountered. If a piece of the new growth was removed and pressed between the fingers, it gave the impression of a piece of firm

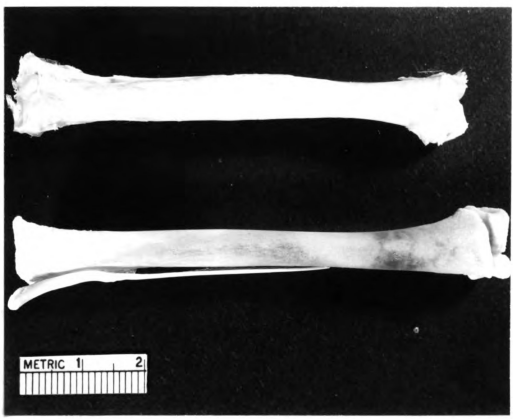

Figure 3. Upper tibia is from a 54-day-old chicken inoculated at 1 day of age. Notice the enlargement of the diaphysis. The lower tibia is from a control bird of the same age.

cheese or caseous material which did not crumble easily, and it had a distinct pale, yellowish color.

Cross section of an affected bone revealed an eccentric appearance of the cortical bone, the affected wall being noticeably thickened while the opposite wall was thin and normal in appearance with the medullary canal appearing to be situated near one edge of the bone rather than in the center (Figure 4).

If the lesion had not completely surrounded the bone, the edges of the newly forming bone tapered to a fine line which blended with the normal periosteum and bone. If it had progressed completely around the shaft, the entire cortical wall was thickened and yellowish in color but one side was always thicker than the other. In the more advanced lesions, the medullary canal was reduced to a small opening. In this experiment, in these early stages of development of the lesion, the medullary canal was always visible but in older, more advanced lesions the medullary canal may be completely obliterated.

On the longitudinal section of bone cut through an advancing lesion, the new growth could be followed from where it tapered from a noticeable elevation to a fine line that merged imperceptibly with the unaffected bone covered by normal periosteum (Figure 5). The contrast in color between the lesion and normal bone was quite apparent on the cut surfaces. The newly formed bone could be broken and crumbled away from the normal bone by slight pressure with the fingernail or a metal instrument.

The long bones of the wings were affected in some chickens and in 1 the sternum had osteopetrotic lesions. Gross lesions were visible in the bones of 11 of 15 birds that were killed on day 43.

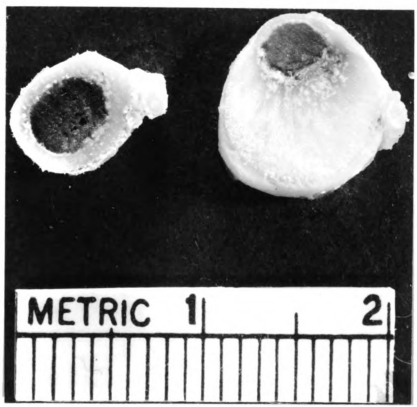

Figure 4. Tibias from 60-day-old chickens. Notice the large osteopetrotic lesion, most prominent on the posterior surface of the tibia on the right, from a chicken that was injected at 1 day of age. Control on left.

Many birds, when they were examined, had fractures of long bones which apparently had occurred spontaneously or were unknowingly caused when they were caught for selection of the birds to be killed each week. At necropsy, many bones were broken from manipulation during dissection (Figure 6).

The spleens in some chickens were atrophic while in others there were no changes. The color and consistency were unaffected. The dimensions of atrophic spleens from 2 experimental birds and 1 control of the same age were compared. The atrophic spleens each measured 8x4x3 and 9x5x5 millimeters compared to 17x11x10 millimeters for the control (Figure 7).

Many of the experimental birds also had erythroblastosis and, early in the experiment, deaths occurred rapidly for awhile because of this condition. In birds with erythroblastosis the livers were enlarged, dark red to mottled and sometimes they were covered by a yellowish, fibrinous pseudomembrane. Ascites was also present.

C. Microscopic Pathology

These findings are summarized in Table 5, page 73.

(a) General Observations

Seventy-three chickens had osteopetrosis. Thirteen of these had microscopic lesions which would have been missed if the diagnosis had been based only on the gross observation. The earliest and smallest lesions that were found microscopically presented 4 distinct characteristics which distinguished the lesion from normal bone. So far as could be determined they all appeared simultaneously. These changes included, (1) a deep basophilic staining of the new osseous tissue

Figure 5. Osteopetrotic lesion on longitudinal and cross section of tibia. Notice how the lesion diminishes in height and merges imperceptibly with the normal periosteum and bone (1). Hematoxylin and Eosin. x 7.

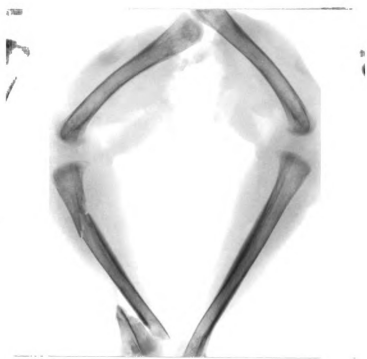

Figure 6. Radiograph showing double fracture of right tibia with displacement of one fracture. Fractures were common in experimental birds.

(2) an increase in size and irregularity of the newly forming Haversian canals, (3) an increase in number and change in position of the lacunae and, (4) a fibrous appearance to the new bone (Figure 8). A fifth characteristic, present in most lesions but absent from a few, was a remarkable increase in the number of layers of osteoblasts in the periosteum.

The early lesions were always located under the periosteum. No endosteal lesions were found where there were not already subperiosteal changes and no endosteal changes occurred until subperiosteal changes were well advanced. The lesions were progressive and proliferative in nature and not degenerative.

In most instances a single focus of osteopetrosis was present on the bone. In a few, 2 or 3 separate foci were found and on 1 bone, 6 small, separate and unrelated foci were found. All of these were subperiosteal and 1 reached from the endosteum to the periosteum (Figure 9). Occasionally a well-developed osteopetrotic lesion was present on 1 side of the diaphysis and on the opposite side a new, very small lesion was found which had just started.

(b) Normal Bone

Normal compact bone was eosinophilic, dense, and homogenous. It had a distinct and uniform structure formed by many Haversian systems. Mature osteocytes lying in the lacunae were deeply basophilic and shrunken from fixation. The lacunae appeared as small, oval to fusiform, open spaces regularly spaces in concentric circles around a small, circular Haversian canal (Figure 10). The periosteum contained 2 layers. The inner layer adjacent to the bone surface consisted of a single row

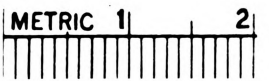

Figure 7. Two atrophic spleens on the right from chickens with osteopetrosis compared to spleen from same age control chicken. Atrophy of the spleen was a common finding in chickens with osteopetrosis. All chickens were 54 days of age.

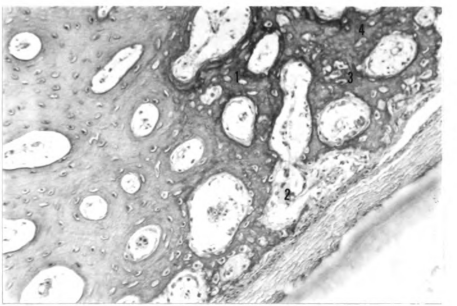

Figure 8. Early lesion which depicts the 4 fundamental changes in an osteopetrotic lesion: (1) basophilic staining of new bone, (2) large irregular Haversian canals, (3) increase in number and size of lacunae, and (4) fibrillar structure of new bone. Normal bone is to the left in the picture. Hematoxylin and Eosin. x 219.

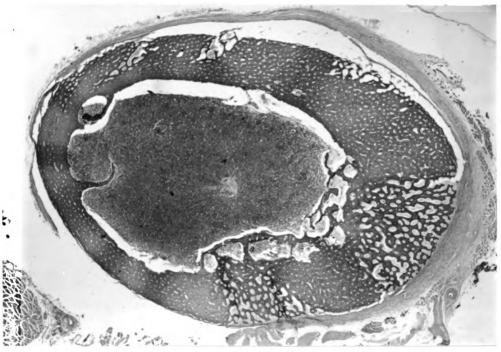

Figure 9. Six separate, subperiosteal, osteopetrotic foci on the humerus. Endosteal changes have occurred at one place. Hematoxylin and Eosin. x 18.

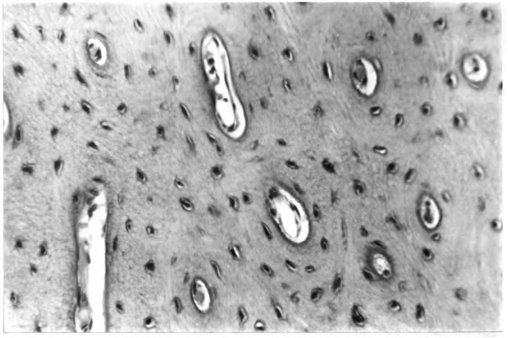

Figure 10. An Haversian system in normal bone with a small round Haversian canal and concentric lamellar rings surrounding it. Hematoxylin and Eosin. x 547.

of osteoblasts. The outer layer was composed of fibrous tissue whose fibers ran in parallel lines with thin elongated nuclei lying along the course of the fibers. The fibers lay in the same plane as the longitudinal axis of the bone. If a new Haversian canal was forming on the surface, the osteoblasts appeared to be increased in number at this point (Figures 11 and 12).

(c) Osteopetrotic Lesions

All osteopetrotic lesions were essentially the same with a few exceptions which will be mentioned. A single description will be made of the lesion in which the progressive development of the microscopic changes will be described starting with an early lesion and progressing to the well-developed stages of the disease.

The newly forming, spongy bone was deeply basophilic. There were no Haversian systems present because of complete disorganization of bone structure. Osteocytes were large and eosinophilic. Lacunae were large, irregular in shape and were present in countless numbers (Figure 13). Many lay adjacent to others and often several opened into adjoining lacunae forming a large open space. The bone tissue appeared as irregular fine fibers with no systematic arrangement. In early lesions adjacent to normal compact bone, these fine, basophilic lines lay between Haversian systems and penetrated to deeper parts of the cortical wall (Figure 14). Haversian canals were greatly increased in size and the shape varied from round to irregular geometric designs (Figure 15). The spaces were separated only by narrow spicules of spongy, fibrous-appearing bone (Figure 15). They were either filled with osteoblasts or contained only a single row around the periphery of the canal, in which

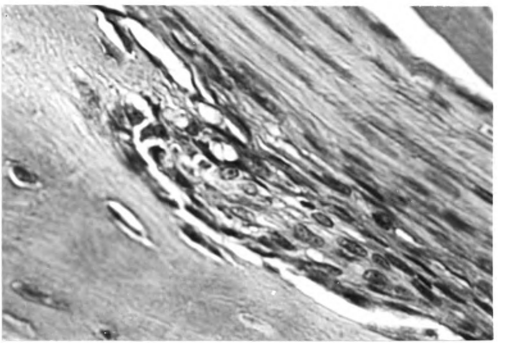

Figure 11. Periosteum and early development of Haversian canal in normal bone. Open spaces are fixation artefacts. Hematoxylin and Eosin. x 957.

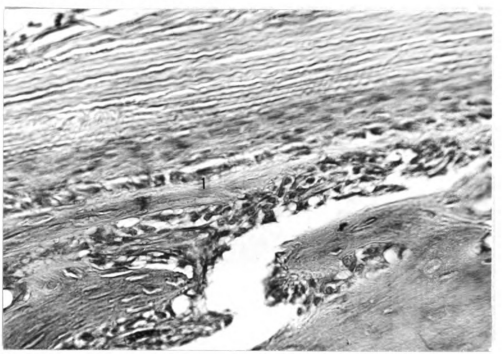

Figure 12. Normal periosteum with a single row of osteoblasts on the bone surface (1). Longitudinal section of Haversian canal in normal bone below. Clear space is an artefact. Hematoxylin and Eosin. x 501.

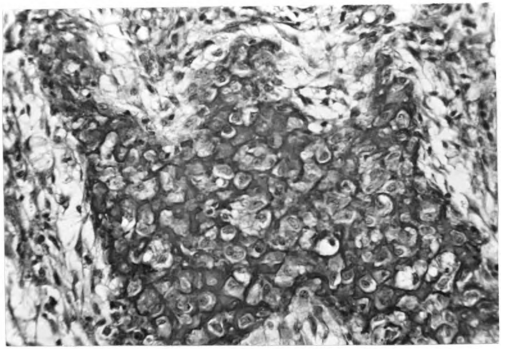

Figure 13. Spongy bone and large, irregular spaces in osteopetrotic lesion. Notice the large, irregular lacunae which are so numerous that they touch and sometimes coalesce. The large spaces are filled with fibrous tissue. Compare with figures 10 and 11. Hematoxylin and Eosin. x 540.

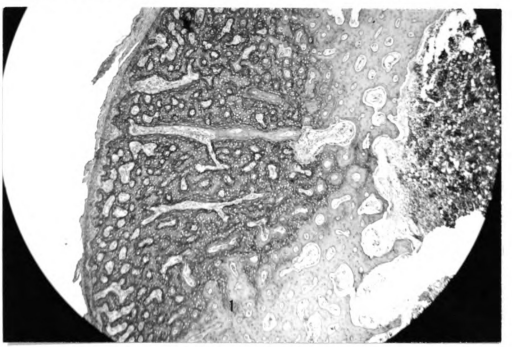

Figure 14. Gradual transition between normal compact bone and basophilic, spongy bone at the margin of an osteopetrotic lesion (1). Hematoxylin and Eosin. x 44.

case they contained increased amounts of a fibrous network of tissue containing a few fibroblasts or modified osteoblasts. There were 1 to several capillaries in each canal. Occasionally a lesion was found in which there was congestion of all capillaries but this was unusual and the same condition occurred in normal bone (Figure 16). Therefore this was considered physiological and not a pathological entity. There was never any hemorrhage or necrosis.

As the lesion progressed, it developed peripherally around the circumference of the bone surface, extended itself toward each end of the bone and at the same time increased rapidly in depth by formation of spongy bone at the surface (Figure 17).

At the margin of the lesion, the newly forming Haversian canals were at once abnormal in shape and size and in the center of the progressive lesion, the canals were altered to a greater degree. Instead of forming a small circular canal, the appearance changed to that of a large, irregular space which sometimes opened into adjoining spaces forming even larger, open, irregular spaces. Between these large spaces were long spicules of fibrous, highly cellular, spongy, new bone which reached to the surface of the lesion under the periosteum. They were capped by a thick layer of osteoid tissue. The fibrous strands and cytoplasm of these highly active osteoblasts covering the tips of the spicules were forced into a convex, saucer-shaped cap because of the extremely rapid growth at these points (Figure 18).

The direction of the canals also changed from running parallel with the longitudinal axis of the bone to a position at right angles to the long axis. In the well-developed lesions this gave the effect of rays of light radiating out from a central focus (Figure 19).

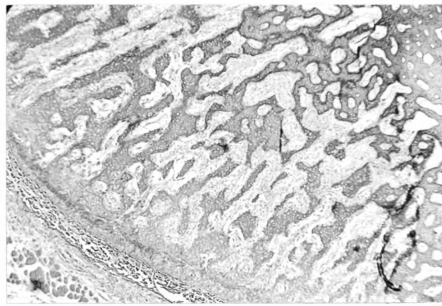

Figure 15. Notice the remarkable irregularity in size and shape of the Haversian spaces in an osteopetrotic lesion. Compare with figures 10 and 11. Hematoxylin and Eosin. x 55.

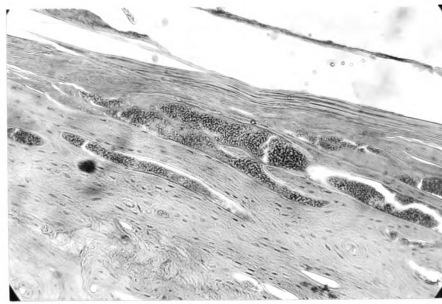

Figure 16. Congestion of capillaries in subperiosteal, normal bone from control chicken. This condition was found in only a few experimental and control birds and was not considered significant. Hematoxylin and Eosin. x 125.

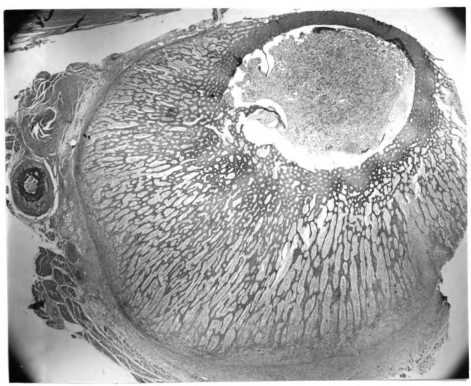

Figure 17. The progressive growth of the lesion both in depth and in extension around the circumference of the shaft is clearly demonstrated. The fibula is completely surrounded by a lesion. Hematoxylin and Eosin. x 16.

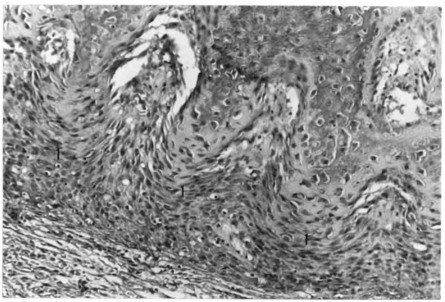

Figure 18. The rapid growth of the spongy bone is depicted by the large amount of osteoid tissue and compression of the osteoblasts at the tips of the bone spicules (1). Hematoxylin and Eosin. x 219.

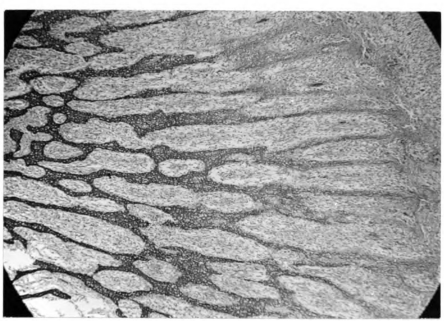

Figure 19. Orientation of the bone spicules and spaces at right angles to the longitudinal axis of the bone. Hematoxylin and Eosin. x 44.

The periosteum was 20 to 30 cells in depth over many of these lesions and was composed of hypertrophic, basophilic osteoblasts (Figure 20). They were fusiform to stellate in shape and cytoplasm streamed from the cells in long fine strands and bundles intermingling with the cytoplasmic processes from adjacent cells. Occasional mitotic figures were found among the cells (Figure 21). Many cells had vacuoles in the cytoplasm so that the nuclei were forced to one edge of the cell (Figure 22). Some osteoblasts contained small,round, homogenous, basophilic bodies in the cytoplasm (Figures 23, 24, and 25). These small bodies usually were surrounded by a halo and 1 to 3 were occasionally seen in the cytoplasm of a cell. They resembled cytoplasmic inclusion bodies described in some mammalian diseases. Degenerating erythrocytes were also present but their staining reaction was much more eosinophilic and they contained nuclear remnants (Figures 25 and 26).

An occasional lesion was found which contained an unusual number of capillaries at the surface of the newly forming bone, but this was only an incidental finding and was not a consistent part of the lesion.

The highly cellular layer of the periosteum was covered with the dense fibrous layer as seen in a normal periosteum. At the margin of the developing lesion the osteoblasts gradually diminished in number until they finally faded out to a normal, single row of cells. The basophilic characteristic of the spongy bone also gradually gave way to normal osteoid and compact bone. The transition from the abnormal osteopetrotic lesion to normal adjacent tissue was so gradual that it almost defied pinpointing the exact place where the lesion ended and normal tissue began (Figure 27). Occasionally the spongy bone ended but

Figure 20. A wide layer of hypertrophic osteoblasts at the surface of a lesion. Compare with figure 12. Hematoxylin and Eosin. x 704.

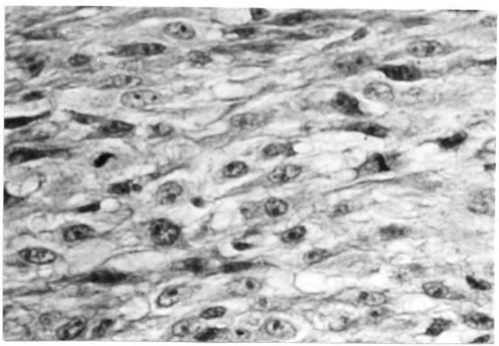

Figure 21. Osteoblasts undergoing mitosis. Mitotic figures were seldom seen, even in these very active lesions. Hematoxylin and Eosin. x 985.

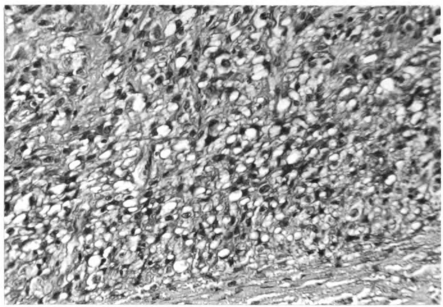

Figure 22. Vacuolation of the cytoplasm of osteoblasts in a subperiosteal lesion. Hematoxylin and Eosin. x 485.

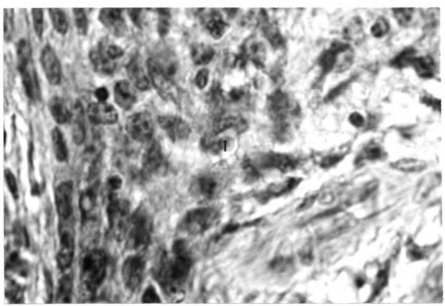

Figure 23. Large, basophilic inclusion body surrounded by a hale in the cytoplasm of an osteoblast (1). Hematoxylin and Eosin. x 1221.

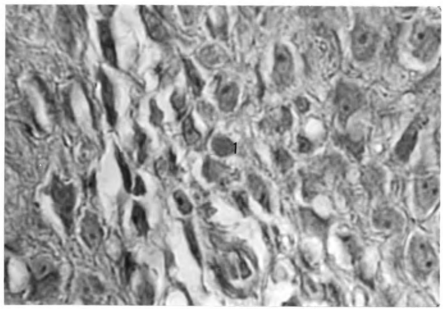

Figure 24. Large, basophilic inclusion body lying free among osteoblasts in the periosteum (1). Hematoxylin and Eosin. x 1221.

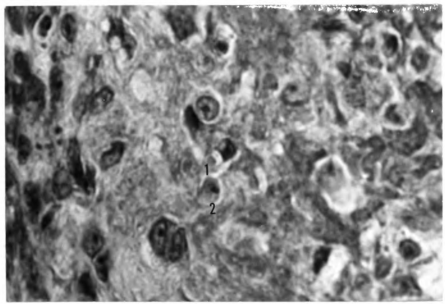

Figure 25. Small, basophilic inclusion body (1) in the cytoplasm of an osteoblast. A degenerating erythrocyte containing a pyknotic nucleus is nearby (2). Hematoxylin and Eosin. x 1221.

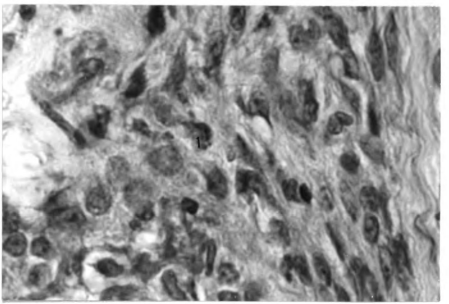

Figure 26. Degenerating erythrocyte with karyorrhexis (1). There is a distinct difference between the inclusion bodies and degenerating erythrocytes. Hematoxylin and Eosin. x 1221.

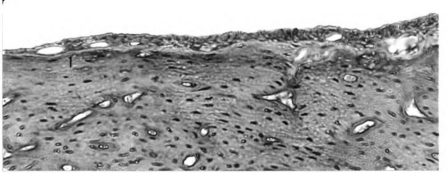

Figure 27. Gradual transition from the margin of a lesion to normal periosteum and bone (1). The transition is so gradual that the point of demarcation narrows to the vanishing point. Compare with figure 5. Hematoxylin and Eosin. x 400.

the hyperplastic periosteum continued beyond the edge of the lesion (Figure 28).

In a few bones, where the lesions had been present for a considerable time, islands of hyaline cartilage had formed. This was not a usual part of the osteopetrotic process, however (Figure 29).

In the advanced lesions, there was a gradual transition between original, normal, dense, cortical bone and the osteopetrotic process (Figure 14). Osteoclasts usually were not found in the lesions. There was no necrosis and no transition to cartilage followed by remodeling of bone. Sometimes the lesion extended from the endosteum to the periosteum. If the endosteal surface was affected long, narrow spicules of spongy bone formed and projected into the marrow tissue (Figures 30 and 31). This caused a marked irregularity in the outline of the medullary canal and probably explained the manner in which the canal was eventually obliterated in advanced lesions. The marrow was sometimes more fibrous than usual; in other instances it had not changed from its usual cellular character.

There were no giant cells in the osteopetrotic lesion and no metastasis to soft tissue was observed.

(d) Muscle

Muscle fibers and bundles in the area of these lesions were undergoing atrophy or were entirely gone and had been replaced by fibrous connective tissue (Figure 32). There were no hemorrhages, no necrosis and no inflammatory cells around the atrophic muscles.

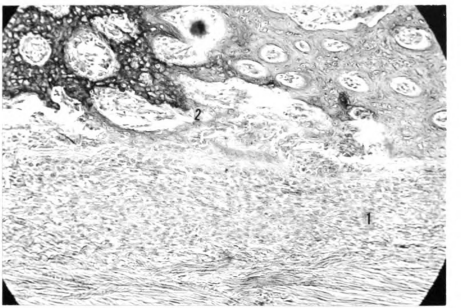

Figure 28. In some lesions the hyperplastic periosteum (1) continued beyond the edge of the osseous lesion (2). Hematoxylin and Eosin. x 128.

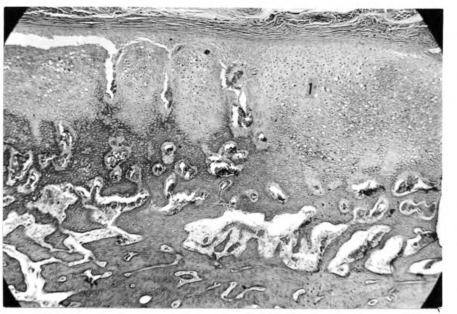

Figure 29. In a few lesions extensive development of hyaline cartilage occurred (1). However, this was uncommon and was not part of the usual development of the osteopetrotic lesion. Hematoxylin and Eosin. x 44.

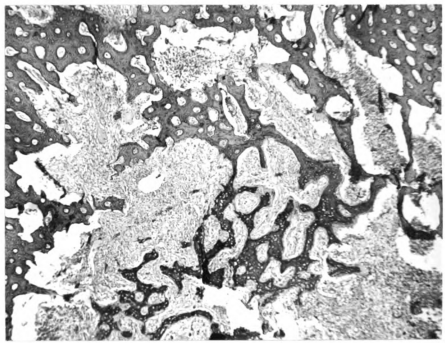

Figure 30. The endosteum and bone marrow were affected only if there was extensive development of the subperiosteal lesion. Fibrosis of the marrow tissue may develop after this. The new endosteal bone is spongy in nature. Hematoxylin and Eosin. x 55.

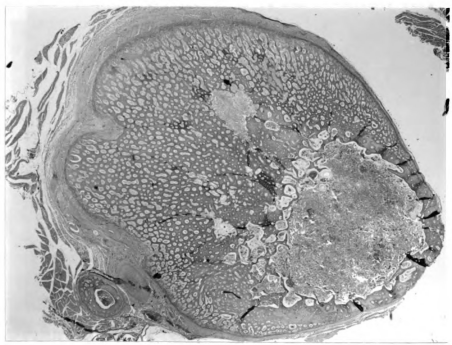

Figure 31. Marked development of osteopetrotic lesion on the tibia with some involvement of the endosteum. Notice the small lesion on the fibula. Compare with figure 30. Hematoxylin and Eosin. x 12.5.

(e) Vascular Lesion

Either arteries or veins or both in the legs, lungs, kidneys, liver and around the adrenal glands contained lesions which partially or wholly obliterated the lumen (Figures 33 and 34). The lesion was formed by the proliferation and growth of the loose, reticular, intimal layer of the vessels (Figure 35). The fibers and cells were somewhat increased in number and in size and the direction of growth was toward the lumen which gradually reduced its size (Figure 36). In the arteries, the growth was sharply delineated at the internal elastic membrane (Figures 33,36, and 37). In some vessels, there were several foci of growths developing at different locations at the same time (Figure 38) and sometimes the adjacent foci had developed to the point they were touching each other (Figures 38 and 39). The growths were covered on the free surface by endothelium; neither inflammatory cells nor thrombosis were present, and in only 2 instances had these growths penetrated the outer structures of the vessel wall (Figure 40). In the liver, some small vessels were entirely closed. In 1 liver, 1 focus was found where a similar kind of tissue was originating from the deep face of Glisson's capsule and penetrating the liver parenchyma. No mitotic figures were found in any lesion and other characteristics of malignancy were absent.

(f) Liver

Some livers contained 2 other lesions unrelated to the 1 just described. These were focal necrosis and bile-duct carcinoma. The necrotic foci were variable in size and not limited to 1 part of a lobule. Some foci were quite small and were located within 1 lobule midway between the periphery and central vein. Others were larger and

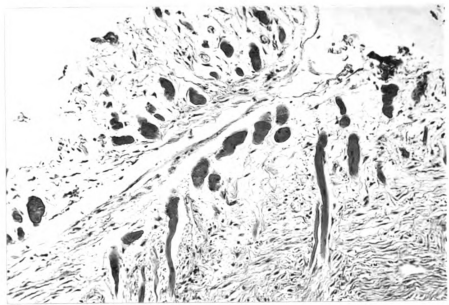

Figure 32. Degeneration and atrophy of skeletal muscles over the lesions followed by fibrous replacement is characteristic of this disease. Hematoxylin and Eosin. x 156.

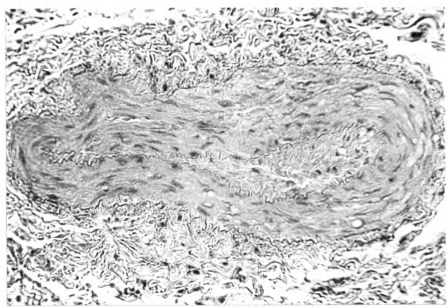

Figure 33. Lumen of a small artery completely closed by intimal growth. Notice that the growth stops at the internal elastic membrane. Hematoxylin and Eosin. x 391.

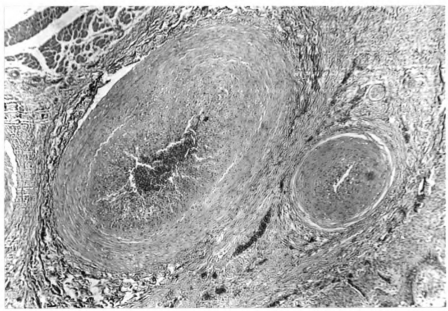

Figure 34. The lumens of two arteries nearly closed by the intimal growths. Hematoxylin and Eosin. x 55.

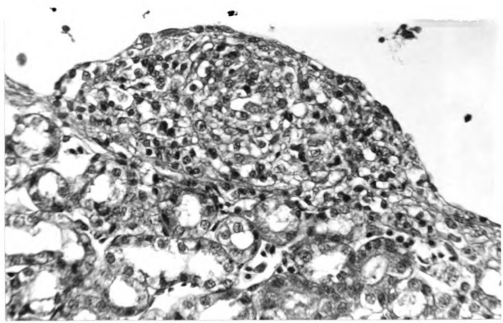

Figure 35. Intimal growth in a vein in the kidney. Cells and fibers are undergoing hyperplasia but mitotic figures are not evident and invasion of parenchyma has not occurred. Hematoxylin and Eosin. x 547.

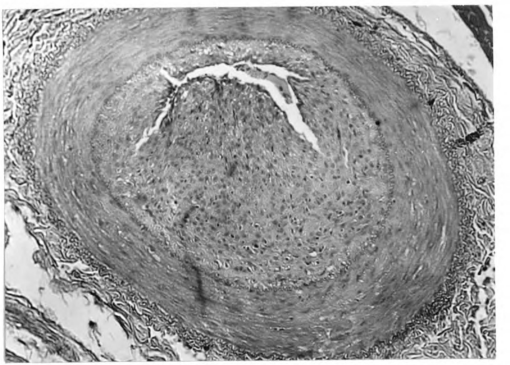

Figure 36. Intimal growth in an artery. The direction of growth was toward the lumen which gradually reduced its size. Hematoxylin and Eosin. x 194.

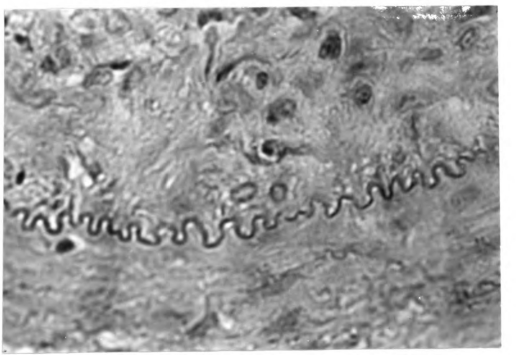

Figure 37. Higher magnification of figure 36, showing the sharp line of demarcation at the internal elastic membrane which separates the intimal growth from the tunica media. Hematoxylin and Eosin. x 1182.

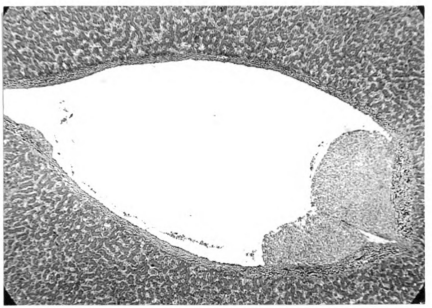

Figure 38. Large hepatic vein with three separate intimal foci, two of which are touching. Hematoxylin and Eosin. x 44.

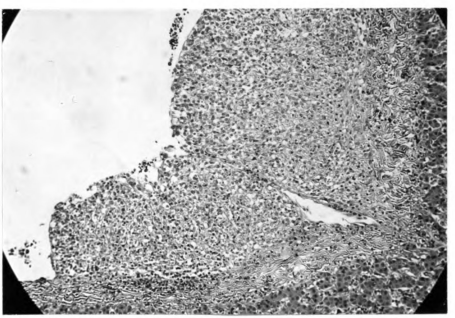

Figure 39. Higher magnification of figure 38. Hematoxylin and Eosin. x 125.

involved parts of 2 adjacent lobules. The hepatic cells had undergone necrosis and remained only as a pale eosinophilic mass (Figure 41). Sinusoids usually were distinguishable and separated parts of the necrotic liver cords from each other. They contained nuclear remnants, cellular debris and erythrocytes. In many instances, erythrocytes were intact even in the centers of the necrotic area. If erythroblasts were present they were even more resistant to destruction. This suggests that either the erythrocytes and erythroblasts moved into the sinusoids after the liver cells died or else they were not susceptible to the causative agent. Hemorrhage was quite marked around the periphery of the necrotic foci.

The bile-duct carcinomas appeared as dense basophilic unencapsulated growths of epithelial cells in the liver parenchyma. A few abortive ducts were forming but most cells were growing in an unorganized arrangement (Figures 42 and 43). Mitotic figures were numerous and invasion of liver parenchyma was apparent (Figure 44).

(g) Spleen

The atrophic spleens did not contain lesions which could account for the atrophy except that they were nearly completely devoid of all lymphocytes and germinal centers. The reticular framework and cells seemed to be increased but this was only from being condensed rather than a real increase. Erythrocytes were present in sinusoids and small arterioles were open.

One significant lesion which was present in many spleens was amyloid. This material was eosinophilic, smooth, homogenous, and foci of it were variable in size. The amyloid was distributed throughout the sections

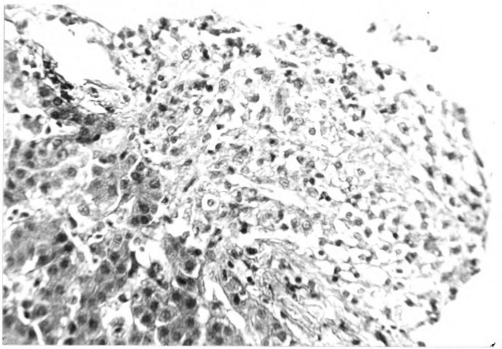

Figure 40. One of 2 instances in which the intimal growth penetrated the tunica adventita. Hematoxylin and Eosin. x 547.

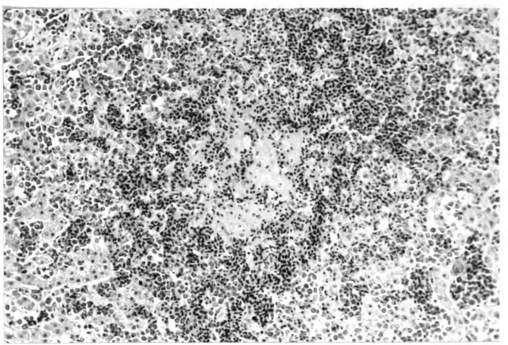

Figure 41. Focal necrosis surrounded by hemorrhage in the liver. This was probably caused by the GAL virus. Hematoxylin and Eosin. x 206.

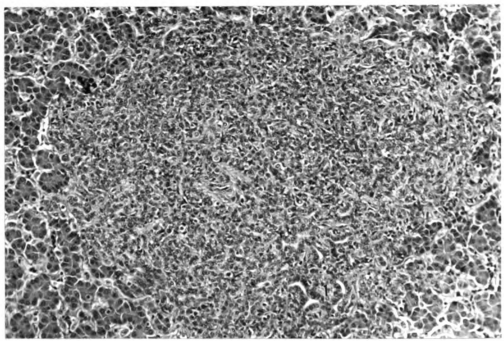

Figure 42. A small focus of bile duct carcinoma with invasion and destruction of hepatic cells. Abortive ducts are forming at one point (1). Hematoxylin and Eosin. x 181.

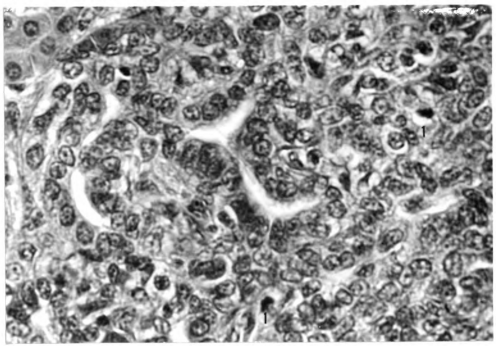

Figure 43. Higher magnification of figure 42 showing bile duct formation and mitotic figures (1). Hematoxylin and Eosin. x 816.

and sometimes formed a thick collar around a small arteriole but at other times was not associated with a vessel. Nuclei and cellular remnants were trapped in spaces in some of the amyloid deposits (Figures 45 and 46).

(h) Lung

The lung in 1 chicken contained a large area of necrosis, cellular proliferation and much fibrin. Large, nonseptate, branching mycelia were found throughout the lesion. These mycelia resembled those of the Phycomycetes and were probably Mucor. This fungus apparently was the cause of the lesion. There were no other respiratory changes except impaction of all capillaries and vessels in some lungs with erythroblasts.

(i) Testicle

The testicle from several chickens had a lesion which was similar in all instances. This consisted of tubular atrophy with the epithelial cells lying unattached in the lumen (Figure 47). They were shrunken and formed a cast although the individual cells were still visible. This atrophy occurred usually at 1 pole or occasionally along 1 edge. It never affected the entire gland. There was never any evidence of inflammation, hemorrhage or trauma to account for the atrophy. In the older lesions, fibrous tissue had grown in from the capsule and filled in the spaces around the atrophic tubules. None of these changes were visible grossly.

(j) Thyroid

The thyroid gland in several chickens had undergone degenerative changes characterized by loss of colloid, partial collapse of follicles and some desquamation of epithelial cells. The cytoplasm of the cells consisted of fine granular debris or interlacing strands of granular

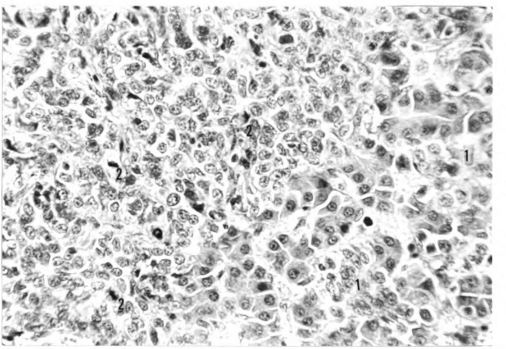

Figure 44. Invasion of hepatic parenchyma by bile duct epithelium (1). Mitotic figures at 2. Hematoxylin and Eosin. x 595.

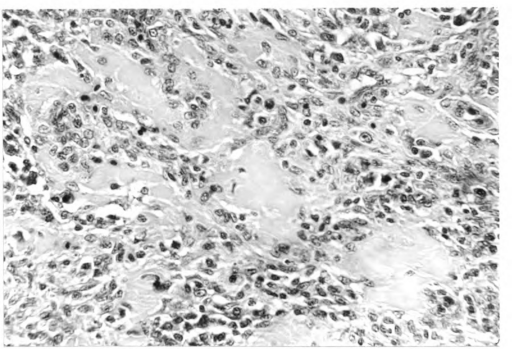

Figure 45. Extensive amyloid deposits in the spleen. Hematoxylin and Eosin. x 532.

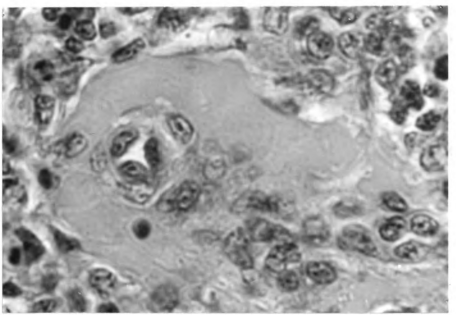

Figure 46. Higher magnification of amyloid around a small vessel in the spleen. Hematoxylin and Eosin. x 1377.

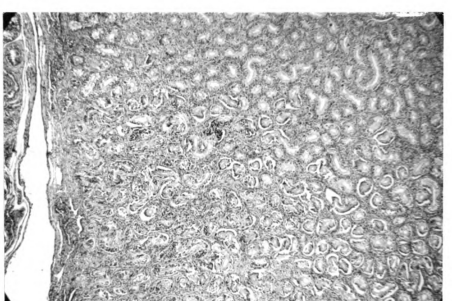

Figure 47. Tubular atrophy in the testicle. Epithelium in atrophic tubules is detached and forms a cast in the lumen. Fibrous tissue is penetrating the area from the tunic. Hematoxylin and Eosin. x 44.

debris. They had the appearance of liver cells filled with glycogen. A few of the nuclei were pyknotic and some had been displaced to the margin of the cell. Some follicles had lost part of the epithelium which had undergone lysis. There was no inflammation or necrosis.

(k) Thymus

The thymus gland occasionally varied in structure but degenerative changes were not present. The usual arrangement in a thymus gland consisted of a dense border of large, deeply basophilic lymphocytes with the center of the gland being open, lightly stained and containing small lymphocytes and Hassel's corpuscles. Occasionally this arrangement was changed and the entire gland contained the small lymphocytes with Hassel's corpuscles distributed throughout the section. The dense border was entirely absent except for small foci of these large, darker cells.

(l) Parathyroid

Parathyroid glands were unaffected except for 1 which contained a cystic space.

(m) Pancreas

Several pancreatic glands contained areas of hyperplasia with no acinar formation. These islands of hyperplastic cells had displaced the normal architecture or had surrounded some acini which were present in the area. In 1 gland, the pancreatic cells were large, had vesicular nuclei, were invading the surrounding normal gland structures and occasional mitotic figures were seen. This was considered an early manifestation of malignant growth (Figure 48).

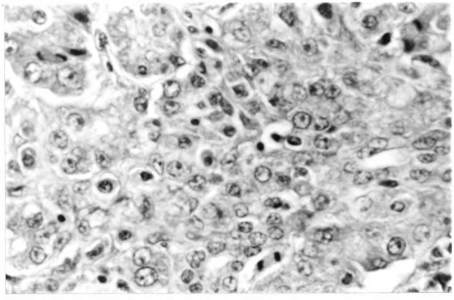

Figure 48. Pancreas from 50-day-old chicken. The large, vesicular nuclei, mitotic figures and lack of organization were considered evidence of early malignancy. Hematoxylin and Eosin. x 632.

In 1 pancreas, many acinar cells were undergoing hydropic degeneration, acinar walls were obliterated or indistinct and the tissue took only a light stain. Islands of normal acini were scattered throughout the section. There was a slight increase in fibrous tissue in the areas of degenerative changes.

(n) Other Tissues

No changes were found in the brain, heart, proventriculus, duodenum or adrenal gland from any chicken in this experiment.

D. Radiological Changes

The entire length of both legs and occasionally a humerus were exposed on the radiograph. Occasionally because of the position of the leg, the fibula happened to lie in the exact plane of the outer surface of the cortex of the tibial diaphysis. This caused the cortical line to appear slightly more dense or blurred in a few instances.

Osteopetrotic lesions were diagnosed in 46 chickens by radiograph compared to 60 at necropsy and 73 microscopically.

With the exception of 1 chicken, no radiographs were made before day 43, the time at which the first group was killed. The single chicken died on day 42, and microscopically osteopetrotic lesions were found on the tibia and femur, but no lesion was seen on the radiograph. Five other chickens which were killed on day 43 had grossly visible lesions of osteopetrosis but no changes were detected on radiographs. These findings indicate that osteopetrotic lesions can be seen grossly before they can be detected by radiograph (Figures 49 and 50).

The early and advanced lesions presented the same appearance except for size. The lesion appeared as an area of increased density with slight

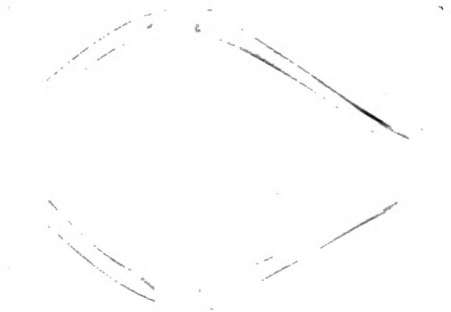

Figure 49. Radiograph of the tibias and femurs from a 43-day-old experimental chicken in which osteopetrotic lesions were visible grossly but could not be seen on the radiograph. Compare with the same age control, figure 50.

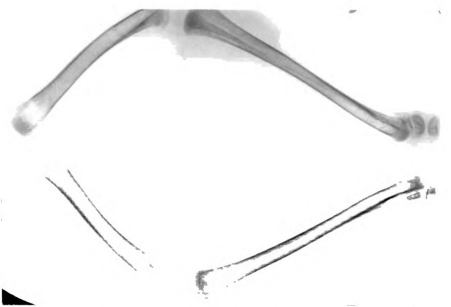

Figure 50. Radiograph of tibias and femurs from control bird, 43 days of age. Compare with figure 49.

thickening of the cortical wall followed by marked elevation above the level of the surrounding normal bone as the lesion progressed. The new osseous tissue developed under the periosteum and on the surface of the original cortical bone (Figures 51 and 52). There was no evidence of rarefaction or bone destruction in the developing lesions.

E. Hematological Studies

Seventy-six chickens were available for hematocrit and hemoglobin studies. They were placed in 1 of 5 groups according to their leukosis status. Group 1 was the control. Group 2 was given the virus but did not develop osteopetrosis or erythroblastosis; therefore, group 2 was comparable to the control group. Group 3 had osteopetrosis; group 4 had erythroblastosis, and group 5 had both forms of the disease.

According to the analysis of variance the hematocrit differences among the groups were highly significant. Groups 3, 4 and 5 had lower hematocrit readings than group 1. Group 2 was not different from group 1. In other words, the variation among these groups was sufficiently greater than the normal variation within groups that these differences would be considered due to the disease and not differences within the population.

The mean for each group was obtained by addition of all values and dividing by the number of observations ($\bar{x} = \frac{\Sigma X}{n}$).

Standard deviation was obtained by substitution of approximate numbers into the following formula: $\sqrt{\frac{1}{n-1}\Sigma(X-\bar{x})^2}$, where n = sample size, X = each observation and $\bar{x}$ = the mean. After standard deviations were calculated the analysis of variance was carried out (Snedecor, 1956; Huntsberger, 1958). These data are summarized in Table I.

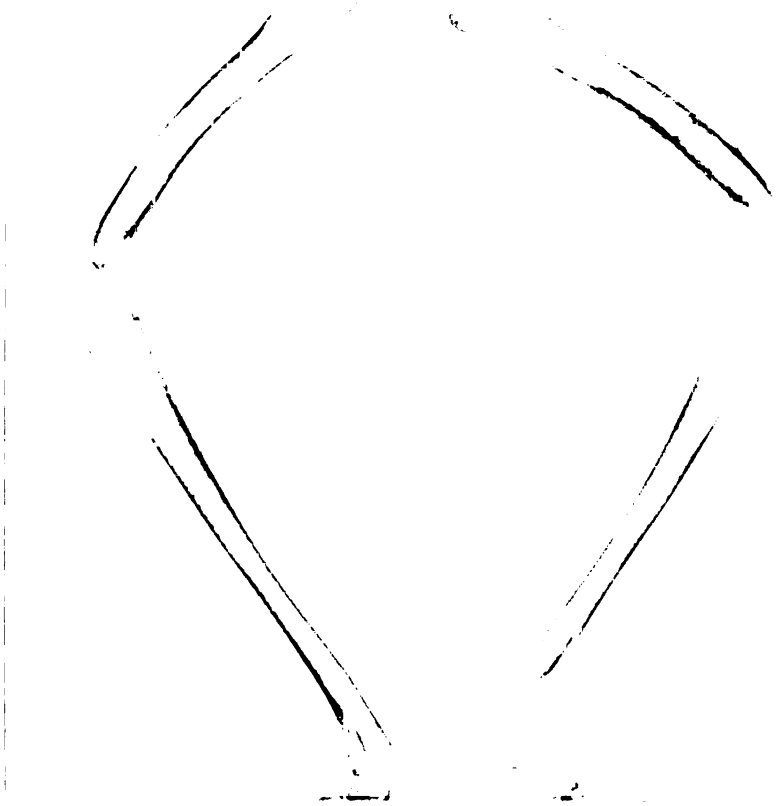

Figure 51. Earliest lesion that was detected by radiograph. The lesion is on the right tibia of a 54-day-old chicken.

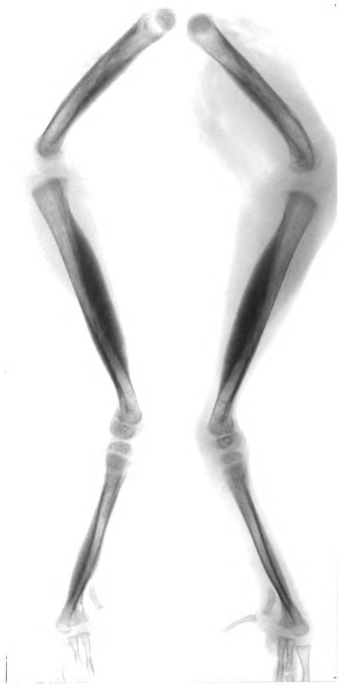

Figure 52. Radiograph showing marked osteopetrotic lesions on the femurs, tibias and metatarsi of a 60-day-old, experimental chicken. Compare with figure 53.

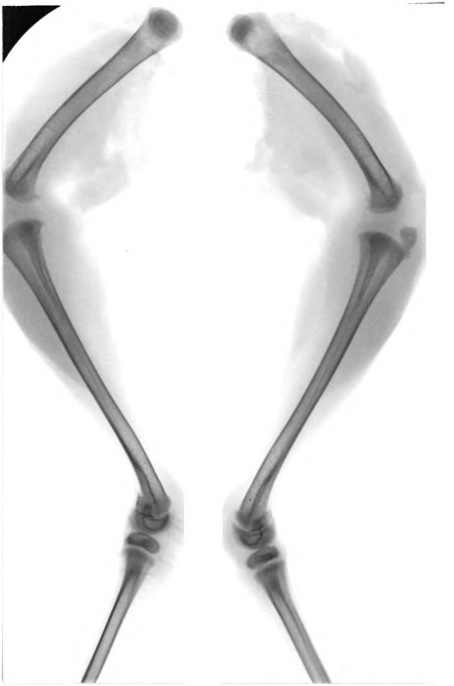

Figure 53. Radiograph of tibias and femurs of 60-day-old, control chicken.

TABLE I. Analysis of the hematocrit readings for 64 experimental and 12 control chickens.

Group	Number of Birds	Mean	Standard Deviation
1	12	28.6	1.7
2	12	28.2	3.3
3	25	24.2	3.4
4	2	26.7	3.5
5	25	24.7	4.4

The variation in hemoglobin levels among the groups was not significant. These data are summarized in Table 2.

TABLE II. Analysis of the hemoglobin levels for 64 experimental and 12 control chickens.

Group	Number of Birds	Mean	Standard Deviation
1	12	22.2	4.2
2	12	22.2	4.2
3	25	22.9	3.0
4	2	24.4	5.5
5	25	23.2	3.6

Seventy-three blood smears were satisfactory for making differential leucocyte counts. These chickens were grouped in the same 5 categories as for the other blood studies and were numbered in order 9, 10, 11, 12 and 13 in the same order for the 5 conditions as given above.

An analysis was performed using a square-root transformation of all counts except lymphocytes to see if there were any significant differences among the groups (Snedecor, 1956). According to the test, there was a significant difference in the monocyte counts of the 3 infected groups, numbered 11, 12 and 13, when compared to the 2 noninfected groups numbered 9 and 10. There also was a difference in the basophil count in the 2 birds in group 13. These data are summarized in Table 3.

TABLE III. Analysis of the differential leucocyte counts for 61 experimental and 12 control chickens.

Group	No. of Birds	Lymphocytes	Monocytes	Heterophils	Eosinophils	Basophils
9	12	85.9	0.83	4.16	3.88	0.19
10	12	81.0	1.56	10.36	2.10	0.04
11	24	74.9	4.08	9.24	3.45	0.26
12	2	70.5	3.92	15.60	3.31	5.47
13	23	80.2	2.52	10.11	1.36	0.73
All	73	79.3	2.46	8.88	2.52	0.37
Significant at		N.S.	.05	N.S.	N.S.	.05

N.S. - not significant.

F. Serum Alkaline Phosphatase

Fifteen serum samples from experimental birds with no osteopetrosis (group 7) and 49 samples from birds with osteopetrosis (group 8) were compared with the serums from the 12 control chickens (group 6). There was no significant difference in the serum alkaline-phosphatase levels among the 3 groups. These data are illustrated in Table 4 (see discussion, p. 85).

TABLE IV. Analysis of the results of the serum alkaline phosphatase levels from controls and chickens with osteopetrosis.

Group	Number of Birds	Mean	Standard Deviation
6	12	14.1	3.7
7	15	13.9	5.4
8	49	13.8	1.2

TABLE V. Summary of lesions that were found in each of the 4 groups of chickens. These groups also include those that died during the half week prior to or following the day on which each group was killed.

Lesion	Group 1, 43 days of age	Group 2, 50 days of age	Group 2, 57 days of age	Group 4, 63 days of age
Amyloid	+	+	+	+
Bile duct carcinoma	-	-	+	+
Erythroblastosis	+	+	+	+
Fractures	+	+	+	+
Hepatic necrosis	+	+	+	+
Inclusion bodies in osteoblasts	-	+	+	-
Intimal growths	-	+	+	+
Mycosis, pulmonary	+	-	-	-
Osteopetrosis	+	+	+	+
Pancreatic carcinoma	-	+	-	-
Parathyroid cyst	-	+	-	-
Splenic atrophy	+	+	+	+
Testicular degeneration	+	+	-	-
Thyroid degeneration	-	+	-	-

+ = Present in 1 or more chickens

- = Not seen

V. DISCUSSION

A. Clinical

The chickens in figures 1 and 2 were from 2 other experiments on osteopetrosis. Birds from this experiment had not developed lesions large enough to be photographed before being killed. Therefore, these other 4 chickens were substituted in order to illustrate 2 different degrees of bone involvement in 2 different ages of birds as well as to show the massive size which the lesions will attain if birds are allowed to live long enough. The contrast between controls and experimental birds also emphasized the difference in growth rates, appearance and feather condition. In figure 1, the birds were 49 days of age. They were from the same hatch and had the same care, feed and type of housing. The control on the right weighed 1350 Gms. and the experimental bird weighed 1080 Gms. The circumference of the left metatarsus of the control measured 4.0 cm. compared to 5.6 cm. for the experimental bird.

The birds in figure 2 were 183 days of age. They were from the same hatch and had always been penned together. The control on the right weighed 2520 Gms. and the experimental bird weighed 1485 Gms. The circumference of the left metatarsus of the control measured 4.7 cm. compared to 10 cm. for the experimental bird. All measurements were made at the center of the diaphysis.

The stilted gait, limping, and preference for sitting down most of the time suggested that the birds were uncomfortable and probably

experienced pain when moving about. However, pressure on the bony lesions did not illicit a response, which fact indicated that the bones were not sensitive. The latter is in agreement with the observation of Jungherr and Landauer (1938).

B. Bone

In speaking of hard-tissue (bone, cartilage and teeth) lesions the fact should be borne in mind that the primary changes must first occur in the cells and tissues forming the periosteum and marrow and filling the Haversian canals, and that reactions of the bone tissue proper are always secondary (Weinmann and Sicher, 1955). Osteoblasts are very sensitive to the action of bacteria and viruses. The viruses of hog cholera, swine influenza, canine distemper and avian encephalomyelitis cause hypoplasia, atrophy and even death of the osteoblast (Monlux, 1961). On the other hand, the severity of the irritant (living or non-living) may not be great enough to cause death of the cells. There may be a minimal response or the cells may be stimulated to reproduce themselves at a rapid rate, in which case the response is called hyperplasia.

In all instances in this study, the earliest microscopic lesions were located under the periosteum and never under the endosteum, and there was never a lesion deep in the cortical bone that did not stem directly from the periosteal lesion immediately above it. No endosteal lesion, marrow involvement or change in the original compact bone was seen without accompanying well advanced subperiosteal lesions. The conclusion here, then, was that the osteopetrotic lesion was basically a function of the infected osteoblasts and the changes in the bone tissue and structure were secondary. This is supported by Weinmann and Sicher (1955).

In other words, the fundamental change occurred in the osteoblast at the time it was parasitized. The change, however, was not visible by the methods used in this study. This fundamental change in the osteoblast caused it to elaborate tissue which was different in several respects from normal bone. These changes were characterized by a difference in staining reaction and structure of the tissue. Eventually, osteoblasts increased in number and size as seen in many of the lesions.

In radiographic studies, Holmes found the earliest lesions under the periosteum, but Bell and Campbell (1961) reported their earliest radiographic lesions were endosteal. Jungherr and Landauer (1938) stated that in the earliest stages, medullary fibrosis, increased osteoclasia and degeneration of old bone were present. These observations were not supported in this study. Osteoclasts were notably absent from the lesions. However, they were present around the trabeculae and endosteal surfaces of nearly all bones indicating that they were present in the normal location but were not part of the developing osteopetrotic lesion. This is merely to say that the osteoblastic activity at this stage in the progressive development of the osteopetrotic lesion was more important than the osteoclastic activity. Medullary fibrosis did not occur until the lesions were well advanced.

It was estimated from the well-developed lesions in a few of these chickens that lesions were present probably by day 30, and microscopically they would have been seen several days before this.

The study of this series of lesions commencing with the smallest and progressing to the largest suggested the following step-by-step development of the lesion. Initially a few osteoblasts were infected by the virus. The time at which this infection occurred was not apparent; but reasoning

from the advanced state of some lesions found in the first birds killed on day 43, the infection could have occurred anytime from 1 to 25 days of age. The irritant (virus) apparently stimulated the osteoblasts but did not kill them.

Under the influence of the irritant (virus) the osteoblasts increased in activity and immediately elaborated large, coarse fibrils that were deeply basophilic in contrast to the eosinophilic character of the adjacent, compact bone. Simultaneously, the superficial indentations that were destined to become Haversian canals in the compact cortical bone were not closed or reduced in size by the laying down of lamellar bone; consequently, they appeared in the new, basophilic, fibrillar matrix as large, open, round to irregular spaces with little resemblance to normal Haversian canals. Immediately thereafter, a noticeable increase in cellularity occurred in the new bone, and osteocytes and lacunae were often so numerous and crowded that adjacent lacunae coalesced and formed larger, open spaces containing several osteocytes. This rapidly forming new bone had a noticeable fibrillar appearance. Finally there was a marked increase in the number of layers of osteoblasts in the proximal layer of the periosteum. These are the 5 characteristics mentioned in the results which distinguished a very early osteopetrotic lesion from normal bone. Because of the extremely rapid formation of osteoid matrix, the trabeculae became oriented to the deep face of the periosteum and appeared as long columns of very cellular, fibrous bone capped at the periosteal surface by a thick layer of osteoid tissue. The large simulated Haversian spaces were formed by the columnar trabeculae and 2 structures together, radiating at right angles to the longitudinal axis of the bone, gave the appearance of the "sun ray burst" described previously and

below. There was no resemblance of this new bone to the regular, systematic organization of normal, compact bone. As the lesion continued to grow, its development was characterized only by an exaggeration of the above steps and not by any change in form or structure. The lesion spread both around the bone and longitudinally toward each end. In a rapidly growing lesion, new spongy bone formed at a rate greater than 1 mm. per week (Sanger, et al., 1963).

It was concluded from this study that the changes which were described in the osteopetrotic lesions developed initially at the time the new bone was formed and did not occur later as a result of remodeling or penetration of the original normal bone by the osteopetrotic process. Osteoclasts were extremely scarce in these lesions and bone remodeling depends upon osteoclastic activity. Since they were very scarce, it seemed reasonable to conclude that whatever changes were seen occurred as the new bone was formed. This is not to suggest that osteoclastic activity might not be present in later stages of the disease.

The course and development of the lesion which has just been described is the usual response of the periosteum to an irritant.

> "The bundles of fibrils in the matrix of immature bone are coarse and irregularly arranged. The osteocytes are numerous, but irregular in shape and arrangement, and have only a few processes. This immature, primitive or coarse fibrillar bone is always arranged in trabeculae and is, therefore, always spongy bone" (Weinmann and Sicher, 1955). "Fibrous bone, in repair, neoplastic, inflammatory and irritative (or reactive) processes is laid down as a rapidly manufacturable material. When the stimulus to fibrous bone production is intense, the trabeculae begin to show uniform orientation, typical examples being the trabeculae in the sun ray burst of an osteogenic sarcoma" (Frost, 1960).

This description of the "sun ray burst" appearance of the newly forming trabeculae is the appearance of the trabeculae and enlarged irregular

Haversian-like spaces in all of the well-developed osteopetrotic lesions in this study. It, also, is the appearance of inflammatory reactions of bones in humans as described and illustrated by Weinmann and Sicher (1955, pp. 351, 355).

Early writers described the condition that is now recognized as osteopetrosis as an inflammatory condition (Bell and Anger, 1924), (Besnoit and Robin, 1922), (Brocket, 1935), Pugh, 1927) before it was associated with the leukosis complex. Burmester et al. (1946), Monlux (1961) and Moulton (1961) described the lesion as one of hyperplasia. Darcel (1960) and Campbell (1961) also considered it a non-neoplastic condition. Neither the cell characteristics nor the nature of the lesion conform to the criteria which are used for the recognition of malignancy (Anderson, 1961).

(a) Fractures

The reason for the many fractures was not apparent. Some of the fractures occurred either spontaneously or when the chickens were handled and were not discovered until the birds were necropsied. Other fractures occurred during manipulation at necropsy in spite of careful efforts to avoid breaking the bones. In some chickens, the fracture was complete with displacement of broken ends. In others the fracture appeared only as a line across the diaphysis with no displacement. On the radiograph no attempt at repair was visible even on the ones with no displacement. Some chickens had fractures but there was no evidence of osteopetrosis on the bones and sections that were studied either grossly, histopathologically or by radiograph. However, osteopetrosis may have been present on some bone that was not examined; therefore, it cannot be concluded that fractures occurred in the absence of osteopetrosis. Controls did not

have any fractures. Radiographs of fractured bones did not reveal any decreased density in the cortical bone when compared to controls of the same age. Gross, et al. (1959) also reported that the bones broke or could be crushed easily. Jungherr and Landauer (1938) and Coles and Bronkhorst (1946) reported that the bones were more resistant to fracture than normal.

(b) Inclusion Bodies

The small objects which were present in the cytoplasm of osteoblasts had the characteristics of inclusion bodies. Sharpless and Jungherr (1961) described intranuclear inclusion in liver cells from chickens infected with the GAL virus. It may be that these bodies were related to the presence of this virus in these chickens. It has also been reported that so-called inclusion bodies are nonspecific such as the "hyaline-droplet" degeneration described by Smith and Jones (1957). It may be that these small bodies were only products of degeneration. If they were degenerative products, they were different from degenerating erythrocytes which had a distinct eosinophilic color and contained nuclear remnants. To attribute them to degenerative changes is not entirely consistent because the osteopetrotic lesions were not degenerative in nature and osteoblasts were not undergoing recognizable degeneration, either those with or without inclusions in the cytoplasm.

C. Intimal Growths

The fibrillar growths which developed in the vessels of the liver, kidney, lung, limbs and in the large artery adjacent to the adrenal gland

did not present any evidence of malignancy. The direction of growth was consistently toward the lumen, the line of least resistance. In only 2 instances, was the growth penetrating the media of the vessel wall. In 1 liver, a similar tissue of fibrllar type appeared to be originating from the deep face of Glisson's capsule. The growth did not cause thrombosis of vessels or necrosis even though some vessels were virtually closed. Collateral circulation must have been sufficient to meet the needs of the tissues, at least in some organs. The intimal growths described here did not form channels, did not contain blood cells or giant cells and were not accompanied by necrosis like the endotheliomas described by Furth (1934). Jungherr and Landauer (1938) reported that portal cirrhosis occurred and also lymphoid infiltration and hyaline degenerative changes in the vascular walls. Gross et al. (1959) found an occasional cirrhotic liver. The lesions which these authors described did not accompany the blood vessel growths reported in this study.

D. Liver and Pancreas

The focal necrosis was attributed to the GAL virus described by Sharpless and Jungherr (1961) which was an inapparent contaminant carried in the RPL 12 inoculum. There were no intranuclear inclusion bodies, however. The early bile-duct carcinomas and pancreatic adenomas were probably caused by the lymphomatosis virus much like those in the kidney described by Burmester et al. (1959) and Walter et al. (1962).

E. Testicle

The reason for testicular atrophy was not apparent unless this was a function of the GAL virus or of the intimal vascular growths. The atrophy was more than a transitory lesion because in several testicles,

fibrous tissue was growing into the gland from the deep face of the tunic and filling the spaces vacated by the tubules. In no instance, however, was there atrophy of the entire gland. Holmes (1961) found infantile testicles in some experimental birds. This condition wasnot part of the lesion described here. Bell and Campbell (1961) reported that the gonads were retarded in development.

Tubular atrophy may also have been primary as a result of ischemia caused by the narrowing of vascular lumens. This, in turn, was then followed by connective tissue growth, or the connective tissue growth could have been primary, being caused by the same thing that stimulated the intimal vascular growths. The crowding from the connective tissue would then, in turn, cause tubular atrophy from pressure as well as interference with blood supply.

F. Thyroid Gland

The degenerative changes in the thyroid glands resembled those described by Gobel (1954) which he thought were caused by thyrotoxins. However, he could not identify the source of the toxins. The photomicrographs in his paper showed degenerative changes in glands which appeared similar to those described in this work. Follicles were partially collapsed; colloid was not present or only partially filled the follicle. Epithelial cells were undergoing degeneration and many were lying free in the lumens. In thse avian thyroids, these cells were considered to be desquamated epithelial cells but Goble (1954) stated that the ones he saw were spherical agranulocytes; this was interpreted to mean they were macrophages although Goble may not have intended to imply this. There was no inflammation and no fibroplasia like that seen in the degenerative

testicular lesion. It may be that this lesion is a reaction to the GAL virus but less severe than that in the liver.

G. Muscle

Muscular atrophy and degeneration probably occurred as a result of 2 conditions acting either singly or in combination. The reduced blood supply brought about by closure of the lumens of the vessels from intimal proliferation would reduce both nourishment and oxygen to the muscles. This would cause atrophy, and continued loss of nutrition would eventually lead to degeneration. The second cause could be attributed to pressure atrophy and degeneration from the increase in bone size from osteopetrosis. This gradual increase in bone size would apply pressure to the muscles gradually reducing their blood supply as well as restricting movement. There would also be incomplete elimination of waste products which, in turn, would aggravate the condition, or both of these conditions could be acting together which did happen in some chickens. These degenerative muscular changes would also account in part for the affected gait and reluctance of the birds to stand.

H. Spleen

Splenic atrophy was a common finding in birds with osteopetrosis; however, it did not occur in every one. The reason for the decrease in size stemmed from acomplete absence of white pulp in the spleen. The other elements appeared normal. There seemed to be a slight increase in the reticular framework but this was more apparent than real because of the concentration of reticulum due to reduced size. Splenic atrophy was reported by Gross (1959), Holmes (1961), and Bell and Campbell (1961. Jungherr and Landauer (1938) reported hyperplasia of the Malpighian corpuscles.

I. Thymus

Absence of lesions in this gland was in agreement with the findings of Bell and Campbell (1961).

J. Parathyroid

The histological appearance of these glands was no different from the controls. The large cystic space in the single gland was not considered significant. Jungherr and Landauer (1938) found significant changes in only 2 glands. One was lymphocytic infiltration associated with generalized lymphomatosis and the second was a small cell hyperplasia associated with secondary osteoporotic lesions. Krook and Barrett (1962) listed 3 morphological ways to evaluate the functional capacity of the parathyroid glands: (a) by weight or volume of the glands; (b) by conventional histological examination of the glands; and (c) by quantative morphological analysis of the glands. They also quoted Eger and Van Lessen and Engfeldt that estimating functional activity based on histological examination was rather inaccurate. Krook and Barrett (1962) further stated that the histological picture of the parathyroid glands of most species is often heterogeneous with transitional cell types. The glands from these chickens were studied only by histological examination. It might be that additional studies would have revealed significant changes.

K. Hematology

The 3 groups of chickens which were suffering from osteopetrosis or erythroblastosis or both diseases had much lower hematocrit readings than the controls or the unaffected experimental birds. However, the circulating erythrocytes contained the normal amount of hemoglobin. The

explanation for the anemic appearance of affected birds in this experiment lies, then, in a deficiency of erythrocytes rather than in reduced concentrations of hemoglobin in the cells.

According to Table 1 published by Bell and Campbell (1961) there was very little change in the hematocrit readings between affected and unaffected birds. They also stated that the red cell counts were unchanged. They found a deficiency of white cells typical of a myelophthisic anemia (granulocytes originating in the bone marrow). They did not report any measurement of hemoglobin levels.

Jungherr and Landauer (1938) reported there was a decrease in hemoglobin in affected birds when compared to controls. They concluded that blood from affected chickens was essentially aleukemic but showed evidence of a mild, nonspecific anemia.

The 3 groups of diseased birds, divided on the basis of the manifestations described above, had a significant increase in monocytes when compared to the 2 groups of uninfected birds. The reason for this was not apparent.

The 2 chickens which had only erythroblastosis had an increase in basophils but the reason for this was not known.

Total leukocyte counts were not made; therefore, it is not possible to say whether the increased monocyte and basophil counts were relative or absolute increases.

L. Serum Alkaline Phosphatase

In the osteopetrotic lesions, the osteoblasts were hypertrophic and hyperplastic and new, spongy bone was formed at a rapid rate. According to Hawk et al., 1954 the rapid formation of normal bone, or new bone

in repair, or calcified and uncalcified pathological bone causes an increase in the level of the enzyme, phosphatase, in the blood. Rapid destruction of bone also causes an increase in the level of the enzyme. Numerous reports in the literature show that chickens have elevated phosphatase levels under varying conditions (Bell et al., 1959; Bell, 1960; Siller, 1959, and Hurwitz and Griminger, 1961). Therefore, it was anticipated that the serum enzyme level would be increased noticeably in these chickens which were suffering from osteopetrosis. However, comparison of the enzyme levels between affected and unaffected birds showed that there was no difference between the 2 groups in this experiment. This was unexpected and the question was raised whether there might have been some error in the test. Correspondence with the Sigma Company, whose test was used, did not clarify the matter. But their offer to test serums from other osteopetrotic and normal birds was accepted.

Their test showed that on serums from 4 osteopetrotic birds, the enzyme levels were approximately twice as high as on controls of the same age. Therefore, it can be stated that serum alkaline phosphatase levels are elevated in some chickens with osteopetrosis. Review of the techniques and procedures revealed that the serums from osteopetrotic birds should have been diluted before testing. It may also be that the spectrophotometer had been improperly calibrated or was not working properly. This part of the experiment needs to be repeated.

M. Work Planned for Future

One of the objectives of this experiment was to determine the earliest date at which osteopetrotic lesions appeared on the bones of chickens which had been given the RPL 12 virus at 1 day of age. This objective was not fully satisfied, the reason being that the time for

killing the first birds was not set early enough. In retrospect it can be seen that a large number of birds would have been needed, and the first group should have been killed not later than the third week of the experiment. An experiment will be designed to answer this question by using a larger number of birds and killing the first group at a younger age.

New spongy bone is formed at a remarkably rapid rate in this disease. It would be desirable to know how fast this bone is formed. An experiment has been planned which will permit accurate measurements of the rate at which the lesion grows.

No references were found in the literature in which the density of the bony substance of these lesions had been measured. A measurement of the degree of mineralization of the various lesions would contribute to the knowledge of the disease. An experiment has been designed to provide this information.

For several years, chickens have been maintained at the Ohio Agricultural Experiment Station (OAES) which carry the causative agent of osteopetrosis. A comparative study of the RPL 12 agent and the OAES agent in producing osteopetrosis has been planned.

Because of the inconsistent results of the serum alkaline-phosphatase test between birds of this experiment and other birds with osteopetrosis, plans have been made to repeat this work.

VI. SUMMARY

Osteopetrosis was experimentally produced by injecting day-old chicks with lymphomatosis virus; clinically, affected chickens were ragged in appearance, stunted in growth and the comb and wattles were pale.

The first group of chickens was killed at 43 days of age. Several had well-developed lesions. The lesions nearly always appeared first at the center of the diaphysis of the tibia at the posterior border of the fibula where it touched the tibia. The color of the lesion was pale yellow compared to a grayish-white appearance of normal bone. The lesion gradually spread around the shaft, toward each end and grew in depth at the center tapering toward each end, giving the affected bone a fusiform appearance.

Microscopically the lesion was typical of an inflammatory periosteal reaction. Osteoblasts were hypertrophic and hyperplastic. The periosteum was 20 to 30 cells thick in some places. Haversian systems did not form. Instead a basophilic, fibrous, cellular, spongy bone formed at a rapid rate on the surface of the original cortical bone. Haversian canals developed as large, open, irregular spaces filled with fibrous tissue. Bone spicules formed rapidly, and both the spicules and spaces were oriented at right angles to the longitudinal axis of the bone. On cross section, the lesion presented a "sun ray burst" appearance.

Osteoclastic activity was not a part of the lesion. The process was proliferative and progressive. Necrosis and degeneration were not present. The characteristics usually associated with malignancy were not evident in the osseous lesions.

Many livers had focal necrosis which was attributed to the GAL virus carried in the inoculum. Some spleens were atrophic and largely devoid of lymphocytes and germinal centers. Many spleens contained amyloid deposits. Many arteries and veins had proliferative lesions formed by rapid growth of the fine, reticular, intimal layer. These growths completely occluded the lumens of some vessels. They were not considered malignant. The testicles had small areas of tubular and epithelial atrophy.

In the liver, foci of neoplastic bile-duct epithelium were present, and in the pancreas, there were neoplastic foci of acinar epithelium.

The hematological studies were limited in significance. The serum alkaline-phosphatase tests were inconclusive.

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