Pathology of Naturally Occurring Vitamin D$_3$-Deficient Rickets in Growing Broiler Chickens in Japan

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Abstract. Bones and parathyroid glands of 48 broiler chickens from 28 to 69 days of age, naturally affected with rickets, were pathologically studied. Main gross lesions were stunting of the skeletal growth, enlargement at the caput costae, hypertrophy of all the bones and enlargement of the parathyroid glands. Histopathologic lesions of the bones were roughly divided into three stages taking into consideration the developmental process. Stage I of the lesions was the initial alteration consisting of mild widening of the hypertrophic zone. In stage II there were marked widening of the hypertrophic zone with considerable endochondral ossification, a moderate degree of endosteal osteogenesis with osteoclastic resorptions in the marrow cavity, and frequent peristeal osteogenesis and demineralization in the cortex. In stage III most portions of the hypertrophic zone were replaced by osteoid trabeculae and the proliferating zone was widened. Endosteal osteogenesis was conspicuous and extensive areas of the marrow cavity were occupied with abnormal osteogenous tissue. All the parathyroid glands examined showed histopathologic features of hyperparathyroidism consisting of swelling and hyperplasia of chief cells. It was concluded that the bone lesions in rickets of growing broiler chickens varied with time and there was a close relationship between formation of the bone lesions and hyperparathyroidism. Etiologically, this condition might be due to vitamin D$_3$ deficiency in the feed.

In previous papers [9, 10], experiments were performed to determine the characteristic lesions in the bone and parathyroid gland of avian rickets due to vitamin D$_3$ deficiency, and to investigate the cause of rickets which occurred from 1974 to 1975 in growing broiler chickens in Japan. The conclusions in the two studies were as follows; bone lesions of vitamin D$_3$-deficient rickets varied with time, the entire bone including the cortical bone as well as the epiphyseal plate and metaphysis was involved, there was a close relationship between the formation of the bone lesions and secondary hyperparathyroidism, and the rickets which occurred in Japanese commercial flocks might be due to a vitamin D$_3$ deficiency.

This paper describes pathologic changes of the bones and parathyroid glands in rickets which occurred in Japanese commercial flocks in 1974, and clarifies one aspect of the condition.

It has been suggested that avian rickets usually results from an error in feed formulation or mixing and therefore is not commonly encountered [11]. Arierasu and Mifune [1] have reported briefly on the pathologic findings of an outbreak of rickets which occurred at the same time as the present cases were being examined.
Materials and Methods

Materials used for this investigation were 48 morbid broiler chickens ranging from 28 to 69 days of age, consisting of 22 males, 14 females and 12 unknowns. They were collected from three poultry farms (A, B and C) from October to December, 1974. The histories of the flocks under study are as follows.

Poultry farm A: Six thousand five hundred day-old broiler chicks purchased from four hatcheries at weekly intervals were reared in windowless houses and fed a certain formula diet. Many birds showing the following clinical symptoms were observed from September to December, 1974. The initial sign, lassitude, with a slight decrease in feed intake, started at about 2 weeks of age. Later, undernourishment, stunting of growth, anorexia, abnormal gait, leg deformity and anemia became predominant. The morbidity was very high and the majority of the birds in each flock were affected, but the mortality was low. Thirty-nine birds ranging from 28 to 69 days of age, consisting of 15 males and 12 unknowns, were collected from 20 flocks for one month and studied. The feed used on this farm was analyzed and was deficient in vitamin D3 content, namely free of it, but no abnormality was seen in either calcium (about 0.8%) or phosphorus (about 0.6%) content.

Poultry farm B: Many flocks varying in age were reared on this farm and two kinds of formula diets were used. In September, 1974, morbid birds occurred in the flocks in which one specific kind of feed was in use. The same clinical signs as those on poultry farm A were observed. However, they appeared earlier (from 1 week of age) and were more severe. The morbidity varied from 50 to 100% in the different flocks at about 30 days of age. The condition disappeared after the feed was changed to the other brand being used on the farm. The birds for this study consisted of seven 30-day-old broiler chickens, which were 6 males and 1 female collected from one flock in October, 1974.

Poultry farm C: Although the history of the flocks on this farm was not observed in detail, stunting of growth was very evident and the body weight of many broiler chickens at 46 days of age was 250 to 550 g (about a half of the normal). The morbidity was 20 to 30% of one flock consisting of 4,000 birds at 6 weeks of age, but no mortality occurred. The chickens were reared in a windowless house and a specific formula diet was used. The cases for this study were composed of two 46-day-old individuals, which were 1 male and 1 female collected in December, 1974.

After postmortem examination, bone tissues and parathyroid glands were fixed in 10% formalin solution. The bone tissues were subjected to electric decalcification. Both tissues were embedded in paraffin and stained with hematoxylin and eosin. Bones examined were femur, tibia, metatarsus, and fifth rib and thoracic vertebra. Histopathologic examination of the parathyroid glands was done in 38 of the 48 chickens.

Results

A. Macroscopic findings

Main gross lesions common in all the birds examined, which were variable in degree, were as follows; stunting of the skeletal growth, enlargement of the epiphyseal regions, thickening of the cortical bone with narrowing of the marrow cavity resulting in hypertrophy of the bone, decrease of the hardness of the bone, and enlargement of the parathyroid gland. The enlargement of the epiphyseal areas was seen in all the bones of the body and was most evident at the caput costae which showed severe beading (Fig. 2). The softest bones occurred in the cases severely affected, and the long bones and vertebrae cut comparatively easily with a boning knife. Both sides of the parathyroid gland were enlarged and each swollen gland was a rice grain to a soybean in size (Fig. 3).

B. Microscopic findings

a) Bone

The essential lesions in all the bones examined were the same in character, but there were some differences in the developmental process of the lesions. Accordingly, the alterations were divided roughly into the following three stages taking into consideration the developmental process (Table 1).

Stage I: This was the initial lesion. It consisted of mild widening of the hypertrophic zone in the epiphyseal plate (Fig. 4). Endochondral ossification with tunnel-
Table 1. Relationship between stages of bone lesions and severity of each histopathologic alteration

<table>
<thead>
<tr>
<th>Histopathologic lesion</th>
<th>Stage of bone lesion</th>
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<tr>
<td></td>
<td>I</td>
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<tr>
<td>Epiphysial plate</td>
<td></td>
</tr>
<tr>
<td>Widening of proliferating zone</td>
<td>-</td>
</tr>
<tr>
<td>Widening of hypertrophic zone</td>
<td>+</td>
</tr>
<tr>
<td>Acceleration of endochondral ossification</td>
<td>++++</td>
</tr>
<tr>
<td>Bone marrow cavity</td>
<td></td>
</tr>
<tr>
<td>Abnormal endosteal osteogenesis</td>
<td>+</td>
</tr>
<tr>
<td>Osteoclastic resorption</td>
<td>+</td>
</tr>
<tr>
<td>Cortical bone</td>
<td></td>
</tr>
<tr>
<td>Osteoclastic resorption</td>
<td>-</td>
</tr>
<tr>
<td>Abnormal periosteal osteogenesis</td>
<td>-</td>
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<tr>
<td>Demineralization</td>
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Remarks.
The symbols show the severity of lesions: + = mild, ++ = moderate, +++ = severe, and -= not observed.

ing of the hypertrophic zone by capillaries was slight only in the diaphyseal side of the epiphysial plate. No abnormality was in architectures of chondrocytes in the widened hypertrophic zone. There was a mild proliferation of osteoblasts along osseous trabeculae in the metaphysis adjacent to the epiphysial plate. Osteoclastic resorptions increased slightly in the metaphyseal osseous trabeculae and along the inner surface of the cortical bone.

Stage II: In the epiphysial plate there was mild widening of the proliferating zone and marked widening of the hypertrophic zone (Fig. 5). Marked endochondral ossification occurred from the diaphyseal side to the deeper area of the widened hypertrophic zone. Eventually, the ossifying zone increased in width, in which there were a large number of irregular-shaped osteoid trabeculae, and many osteoblasts and chondroclasts (Fig. 6). No morphologic abnor-

mality was in each chondrocyte of both widened proliferating and hypertrophic zones. The endochondral ossification started with tunneling of the hypertrophic zone by capillaries accompanied by chondroclasts, osteoblasts and fibrous connective tissue. Then osteoid seams were formed beneath the active osteoblastic layer and the hypertrophic zone was gradually replaced by newly formed trabeculae containing osteoid.

Abnormal endosteal osteogenesis (Fig. 7) occurred fairly actively along with the preceding endochondral ossification. It proceeded from the metaphysis to the diaphysis, especially along the inner side of the cortical bone. In the cortical bone there were osteoclastic resorptions, demineralization and abnormal periosteal osteogenesis. The osteoclastic resorptions were frequent on the inner surface of the cortical bone (see Fig. 16) and occasionally in the inner region of the cortical old bone (see Fig. 17). Such resorptions occurred often around the Haversian canals. The demineralization was observed in many birds. It was recognized as the presence of osteoid borders on the walls of the Haversian canals in the cortical old bone (Fig. 8). The abnormal periosteal osteogenesis (Fig. 9), which was moderate in degree, was seen in about a half of the birds in this stage.

Stage III: In the epiphysial plate almost the same changes as those in stage II were frequent in this stage. In many cases, however, the widened hypertrophic zone was almost completely spongified as a result of accelerated endochondral ossification and became markedly narrow (Figs. 10 and 15). Conversely, the proliferating zone increased moderately in width and the epiphysial plate was composed mainly of widened proliferating zone.

Both abnormal endosteal osteogenesis
A relationship between the age of the birds examined and the stages of bone lesions is shown in Fig. 1.

b) Parathyroid gland

Histopathologic changes of this organ were almost the same in character in all the 38 birds examined, although there were some variations in degree. A relationship was not demonstrated between the stages of bone lesions mentioned above and the degree of alterations of the parathyroid gland.

The most characteristic changes were swelling and hyperplasia of the parenchymal cells with an increase in mitotic figures (Figs. 18 and 19). These alterations occurred in the majority of the 38 birds. The swollen parenchymal cells had large pale, round nuclei and were rich in cytoplasm which was stained poorly with eosin. The cellular contour was indefinite, ranging from oval or polyhedral to columnar. The swollen parenchymal cells were usually associated with mitotic figures in the proportion of one or two cells in a few lobules (Fig. 19). Rarely karyopyknosis was seen, especially in cases showing markedly swollen cells. Furthermore, there were scattered odd spindle-shaped atrophic parenchymal cells.

The second most characteristic alteration was a cord-like arrangement of the parenchymal cells (Fig. 20). Such changes were prominent in 10 of the 38 birds. The arrangement was slender and winding and there were broad spaces formed between the cords and stroma. The cellular elements showing this arrangement consisted of slightly swollen parenchymal cells which were rich in eosinophilic cytoplasm and had moderately pale nuclei. The cytoplasm had frequently minute vacuoles. Rarely mitotic figures were demonstrated. Atrophic cells were occasionally seen in the
arrangement. Within the spaces formed there was rarely a fibril-like or homogene-
yous, amorphous substance which was stained poorly with eosin. The stromal
connective tissue was frequently loose, and sometimes associated with a slight degree
of lymphocytic and plasmacytic aggregations. Swelling and hyperplasia of the
parenchymal cells mentioned above co-
existed frequently in the sections revealing
the cord-like arrangement.

A proliferation of stromal connective tis-
tue was observed in a few cases (Fig. 21).
In them the lobules were small and com-
pared of parenchymal cells which had a
moderate amount of cytoplasm stained
deply with eosin and round nuclei stained
comparatively darkly with hematoxylin.

Discussion

The pathologic changes of both bones
and parathyroid glands in this study are
identical with those seen in previous ex-
perimental investigations of vitamin D3-
deficient rickets [9, 10], and also resemble
those described in the disorders due to de-
ciciencies in calcium and vitamin D and
lack of sunlight in growing chickens [4, 5,
12, 13]. Moreover, the pathologic features
of the present cases are similar to those of
bone dysplasia of broiler chickens [7, 8] in
many respects.

From the developmental process of the
bone lesions in this study, it was reaffirmed
that changes of the epiphyseal plate varied
with time, while the abnormal endosteal
osteogenesis was progressive throughout.
Accordingly, the endosteal osteogenesis as
well as alterations of the epiphyseal plate
should be regarded as the most characteris-
tic lesions of rickets in growing chickens.
The former seems to indicate the extent of
this disorder irrespective of age.

The extensive abnormal endosteal osteo-
genesis was usually associated with remark-
able osteoclasia. Possibly this results from
secondary hyperparathyroidism as Gratzl
and Köhler [3] and Groth [4] have postu-
lated. Avian rickets due to deficiencies in
calcium or vitamin D accompanies usually
secondary hyperparathyroidism; the so-
called “classical form” of rickets described by
Nairn and Watson [11]. In studies on
metabolic acidosis in the vitamin D-defi-
cient chick, it has been suggested that there
is continued secondary hyperparathyroidism
[2]. It may be emphasized that in vitamin
D3-deficient rickets, bone lesions resulting
from the hyperparathyroidism appear from
the early stages and are predominant.

The abnormal periosteal osteogenesis, on
the other hand, was severe and frequent in
the young birds in this study, and only mild
and occasional in the aged. As physiologi-
cal periosteal osteogenesis in the normal
broiler growing chicken continues until 35
days of age [6], the abnormal periosteal
osteogenesis might have much to do with
the age of the affected birds. It is signifi-
cant that the osteoclastic resorptions proba-
bly resulting from the hyperparathyroidism,
have been present in the inner region even
in the cortical bone in the maturing stage.
These findings suggest that the relationship
between the developmental process of the
bone lesions and the age are important in
understanding rickets of growing birds.

The demineralization observed in the
cortical bone in the present cases resembles
that seen in mammalian osteomalacia. The
changes of both metaphysis and diaphysis
mentioned above are identical with those
in osteodystrophia fibrosa of mammals.
These findings emphasize that the altera-
tions occur simultaneously in certain stages
of avian rickets and that the bone lesions
are very complicated.

Histopathologic pictures of the parathy-

roid gland in this study are the same as those seen in previous experimental investigation [9]. There were two types of swollen parenchymal cells; greatly swollen cells and lesser swollen ones. The former cells may be the most hyperactive ones and the latter be in a regressive stage. In addition, it was demonstrated by electron-microscopic examination that there were some differences in the activated morphology of the parenchymal cells in the present birds [4].

As stated earlier, the diet used on poultry farm A was sufficient in calcium and phosphorus contents and deficient in vitamin D₃ content. Outbreaks of the disorder were prevalent on other poultry farms during a similar period. From this, it is concluded that the rickets in this study might be due to a simple vitamin D₃ deficiency and omitting vitamin D₃ from the premix might have resulted in the deficiency as some other workers have pointed out [11, 15].

References

Fig. 2. Gross lesions of vertebrae and ribs from 30-day-old chickens (the left bird is the control of the same age). There are beading at the caput costae, hypertrophy of the bone and stunting of the skeletal growth.

Fig. 3. Marked enlargement of the parathyroid glands (arrows) from a 30-day-old chicken.

Fig. 4. Longitudinal section through the distal end of tibia from a 32-day-old chicken (stage I of bone lesions). The hypertrophic zone is increased moderately in width without endochondral ossification. The proliferating zone is normal (upper area). Hematoxylin and cosin staining (H-E) ×50.

Fig. 5. Longitudinal section through the distal end of tibia from a 35-day-old chicken (stage II). The hypertrophic zone is greatly increased in width. Invading capillaries accompanied by many osteoblasts, some chondroblasts and a small amount of osteoid, endochondral ossification, extend into the deeper area of the hypertrophic zone. The proliferating zone is increased slightly in width (upper area). H-E ×48.

Fig. 6. Longitudinal section through the distal end of tibia from a 30-day-old chicken (stage II). There are extensive osteoid trabeculae as a result of accelerated endochondral ossification. H-E ×144.

Fig. 7. Longitudinal section through the distal metaphysis of tibia from a 39-day-old chicken (stage II). There are a moderate proliferation of osteoblasts and fibrocytes and an apposition of osteoid seam around osseous trabeculae. Osteoclastic resorptions are scattered along old bone. H-E ×114.

Fig. 8. Cross section through the tibial diaphysis from a 30-day-old chicken (stage II). Osteoid borders (pale areas) resulting from demineralization are clearly seen on the walls of Haversian canals. H-E ×114.

Fig. 9. Longitudinal section through the posterior part of the metatarsal diaphysis from a 47-day-old chicken (stage II). There is abnormal periosteal osteogenesis consisting of a proliferation of osteoblasts in the subperiosteum and a new formation of osseous trabeculae with osteoid seam and an osteoblastic layer. Fibrocytes are present among the trabeculae. H-E ×58.

Fig. 10. Longitudinal section through the proximal end of metatarsus from a 57-day-old chicken (stage III). The epiphyseal plate consists mostly of a remarkably widened proliferating zone, in which the arrangement of chondrocytes is disordered. The hypertrophic zone is very narrow with a mild degree of endochondral ossification (lower area). H-E ×48.

Fig. 11. Longitudinal section through the distal
metaphysis of tibia from a 50-day-old chicken (stage III). There is a marked proliferation of osteoblasts, osteoclasts and fibrocytes, rarely with osteoid. Osseous trabeculae are mostly resorbed and remained as fragments (arrows). H.E. ×228.

Fig. 12. Longitudinal section through the distal metaphysis of tibia from a 50-day-old chicken (stage III). Osseous trabeculae are completely resorbed and replaced by bundles of osteoblasts with some osteoclasts. Fibrocytes and marrow cells are scattered among the trabeculae. H.E. ×240.

Fig. 13. Longitudinal section through the distal metaphysis of tibia from a 50-day-old chicken (stage III). An extreme proliferation of osteoblasts is seen showing a trabecular-like arrangement with some osteoclasts. Marrow cells are scattered. H.E. ×162.

Fig. 14. Longitudinal section through the tibial diaphysis from a 30-day-old chicken (stage III). Osteoclastic resorptions and abnormal endosteal osteogenesis occur on the inner surface of the cortical bone (lower half). There is dilation of developing canal systems with demineralization in the cortical bone (upper half). H.E. ×114.

Fig. 15. Longitudinal section through the distal end of tibia from a 46-day-old chicken (stage III). The epiphyseal plate consists mostly of slightly widened proliferating zone. Abnormal endosteal osteogenesis is extremely marked, so that extensive areas of the marrow cavity are occupied by the osteogenous tissue. The posterior part of the cortical bone (right side) is porous. H.E. ×4.5.

Fig. 16. Cross section through the tibial diaphysis from a 62-day-old chicken (stage III). Extensive areas of the marrow cavity are occupied by abnormal osteogenous tissue containing newly formed osseous trabeculae. Abnormal periosseal osteogenesis is fairly matured around the original cortex. H.E. ×9.

Fig. 17. Longitudinal section through the tibial diaphysis from a 69-day-old chicken (stage III). Multiple osteoclastic resorptions, within which there is osteogenous tissue, occur in the inner region of the cortical bone. H.E. ×32.

Fig. 18. Parathyroid gland from a 50-day-old chicken. Many parenchymal cells are markedly swollen and hyperplastic. H.E. ×340.

Fig. 19. Parathyroid gland from a 30-day-old chicken. Many parenchymal cells are swollen and hyperplastic with mitotic figures (arrow). Somewhat smaller parenchymal cells with prominent eosinophilic cytoplasm are seen in groups. H.E. ×680.

Fig. 20. Parathyroid gland from a 57-day-old chicken. The parenchymal cells show a cord-like arrangement. Irregular-shaped spaces are formed between the arrangement and stromal connective tissue. The cellular elements consist mainly of somewhat swollen parenchymal cells with eosinophilic cytoplasm, sometimes associated with atrophic cells. H.E. ×410.

Fig. 21. Parathyroid gland from a 59-day-old chicken. A proliferation of stromal connective tissue is fairly marked, so that the lobules are small. The parenchymal cells are moderate in size and the cytoplasm is stained deeply with eosin. H.E. ×410.