Histology of the Developmental Process of the Femoral Diaphysis and Its Pathological Significance in Broiler Chickens

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Abstract. Investigation was carried out on the developmental process of the femoral diaphysis in broiler chickens ranging from 1 to 90 days of age.

The diaphysis was formed mainly by apposition of newly formed bone tissue resulting from periosteal osteogenesis around the embryonic bone. Endosteal osteogenesis for the diaphysis, on the other hand, was merely concerned with modification of the inner surface. The bone tissue reached the maturing stage at 55 days of age at the earliest. The feature of maturity was recognized at 70 days of age or later. During the developmental period, especially in the active stage, there were distinct differences in the degree of development and thickness between the anterior and posterior parts of the diaphysis.

Based on these findings, discussion was made on several diseases and lesions which are characterized by abnormal periosteal and endosteal osteogeneses.

Itakura and co-workers [2–5] have studied bone dysplasia in young broiler chickens. This condition was characterized by an abnormal development of bone tissue. No satisfactory discussion has been developed on the relationship between the lesion and the normal developmental process of bone tissue, because there was little literature available on the histology of bone in any avian species.

This article describes the developmental process of the femoral diaphysis in broiler chickens, with special emphasis put on the relationship between this process and the age of the bird. In addition, discussion is made on the relationship between the normal and pathological development of bone tissue, quoting several bone diseases which show abnormal bone formation [1–5, 7, 8].

Materials and Methods

Materials used for this investigation consisted of 57 broiler chickens of both sexes (male White Cornish and female White Plymouth Rock hybrids) ranging from 1 to 90 days of age. Throughout the experimental period, they were reared in batteries and fed a non-medicated diet. Three birds were sacrificed at random for necropsy at 5 days' intervals over a period from 1 to 90 days of age. Clinically or macroscopically, no abnormality was observed in any of the experimental birds.

Both left and right femora from each bird were fixed in 10% formalin solution. After electric decalcification, cross and longitudinal sections were made in such manner that the former might include the middle part of the diaphysis and the latter both anterior and posterior parts. Bone tissue blocks were embedded in celloidin or paraffin and cut into sections, which were stained with hematoxylin and eosin.
Results

The histological findings obtained on the development of the diaphysis were almost the same as those described in many textbooks of mammalian histology. Hence, the minute descriptions are abbreviated here.

Two kinds of osteogenesis, periosteal and endosteal, were observed. Each process started with proliferation of the periosteum, which was followed by the formation of new osseous trabeculae, canal systems (primitive haversian systems), and osteoid tissue in the order listed. Consequently the diaphyseal bone was completed by the formation of outer and inner basic lamellae. The relationship between the age of the bird and the development of the diaphysis is mentioned below in detail. It is summarized in Table 1.

1. Periosteal osteogenesis (Figs. 1 to 6)

   Birds 1 day of age: There was only a slight proliferation of the periosteum in the anterior part of the diaphysis. In the posterior part osteoblasts were numerous in the proliferated periosteum. Simultaneously, the formation of new osseous trabeculae was started around the embryonic bone (Fig. 1).

   Birds 5 and 10 days of age: The formation of osseous trabeculae occurred apparently in the anterior part of the diaphysis. New canal systems began to be formed in the posterior part (Fig. 2).

   Birds 15 to 50 days of age: The formation of canal systems was predominant (Figs. 3 and 4), whereas that of osseous trabeculae declined in both anterior and posterior parts.

   Birds 35 days of age: The formation of osseous trabeculae decreased in the anterior part, but progressed in the posterior part. Canal systems were formed still actively in the anterior part, but reduced in the posterior part.

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<th>Table 1. Relationship between age of bird and bone development</th>
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<tr>
<td>Age of each group (days)</td>
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<td>Periosteal osteogenesis</td>
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<td>Proliferation of periosteum:</td>
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<td>Formation of osseous trabeculae:</td>
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<td>Formation of canal systems:</td>
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<td>Endosteal osteogenesis</td>
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<td>Formation of osseous trabeculae</td>
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<td>Formation of inner basic lamellae</td>
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Remarks.

*: The figure indicates the number of birds with predominant histological changes among three birds examined in each age group.
Birds 40 to 50 days of age: Osteoid tissue was formed continually beneath the osteoblastic layer in both anterior and posterior parts (Fig. 5).

Birds 55 to 70 days of age: Outer basic lamellae were formed progressively in both parts, some of which were completed (Fig. 6).

2. Endosteal osteogenesis (Figs. 7 and 8)
   There was no apparently significant difference in osteogenesis between the anterior and posterior parts.

Birds 1 to 35 days of age: There was no proliferation in the endostem.

Birds 40 to 50 days of age: The endostem proliferated slightly and osteoid tissue was formed a little.

Birds 55 to 65 days of age: Osteoid tissue was formed predominantly.

Birds 70 to 90 days of age: Inner basic lamellae began to be formed, some of which were completed (Fig. 8).

3. Summary of findings of osteogenetic process
   The bone tissue of the diaphysis reached the maturing stage of development at 55 days of age at the earliest. After that, outer basic lamellae began to be formed. In this way, features of maturity of the bone development were recognized at 70 days of age or later.

During the period of bone development, there was a distinct difference in thickness between the anterior and posterior parts of each diaphysis. Namely, in the active stage of development (at 5 to 35 days of age), the posterior part was three to four times as thick as the anterior one. The posterior part was 2.3 times thicker in the maturing stage (at 55 to 65 days of age) and 1.3 times thicker in the mature bone (at 70 to 90 days of age) than the anterior part. On the other hand, when the developmental process was studied comparatively, female birds tended to reach maturity a little earlier than male.

In brief, the mature femur was a simple tubular bone and its posterior part was somewhat thicker than its anterior one. The bone itself was formed mainly by apposition of bone tissue newly formed by periosteal osteogenesis around the embryonic bone. On the other hand, endosteal osteogenesis was concerned chiefly with modification of the inner surface of the diaphyseal bone and formation of osseous trabeculae within the medullary cavity.

Discussion

Inoue [1] gave a brief account on normal histogenesis of the chicken femur in his paper on the medullary bone. He mentioned the developmental process of this bone which was essentially the same as reported in the present paper. He did not refer, however, to the relationship between the age of the bird and the histogenesis of the bone. Pratt [6] studied the development and maturation of the femur of the fowl, stressing the importance of the arrangement of fibres in the bone matrix. In his study, the newly formed matrix of periosteal bone had a homogeneous appearance at 120 days of age and later, and endosteal bone was formed completely at 70 days of age.

Itakura and Yamagiwa [2, 3] reported a new bone disease called "bone dysplasia" in broiler chickens. In this disease both abnormal periosteal and endosteal osteogeneses were obviously in progress and no outer basic lamellae had been formed as yet in any bone of the body even after affected birds (120 days of age) reached the maturing stage of bone. In their next paper [4] they examined broiler chicks characterized clinically by bowleg. In these birds an abnormal focal development of bone tissue occurred in the diaphysis of the leg bone, especially in its middle part, so that the cortex increased in thickness. As lesions
were produced by the formation of new canal systems, it is probable that they may have resulted from abnormal periosteal osteogenesis.

Such abnormal focal periosteal osteogenesis was observed in the leg bones of broiler chickens affected with dyschondroplasia [5]. It was composed chiefly of the formation of new osseous trabeculae, canal systems and osteoid tissue. It can be said that this lesion have resulted from a delay in maturity of the bone tissue, because the affected birds were 10 weeks of age.

Yamagiwi et al. [8] histologically examined the femora of 261 chickens selected at random and ranging from 2 to 162 days of age in order to find the initial lesion of thick-leg disease. In consequence, abnormal extramedullary bone formation (abnormal periosteal osteogenesis) was recognized in only one White Leghorn 93 days old. As this lesion was focal in extension, it apparently had nothing to do with thick-leg disease. Inoue [1] displayed photomicrographs showing abnormal focal hyperplasia of periosteal bone tissue taken from two chickens 108 and 183 days of age, respectively. He suggested that this lesion might have no relationship with the medullary bone.

The histogenesis of thick-leg disease [7] is interesting, although this disease is different in appearance from bone dysplasia. This condition occurs after the bone reaches the maturing stage of development. The essential lesion of the disease was described as intramedullary neoplastic bone formation which had been originated from the periosteum. At the same time occurred extramedullary neoplastic bone formation, which had resulted from abnormal periosteal osteogenesis. The authors reporting this disease stated that such lesion should belong to tissue malformation.

At any rate, it should be noted that both periosteal and endosteal osteogeneses were present in the normal development and the pathological condition of the bone. In addition, it should be emphasized that there was a close relationship between the age of the bird and bone development, and that there was also a difference in the development and maturation between the anterior and posterior parts of the femoral diaphysis. These facts should be borne in mind when histological examination is carried out on avian bone diseases.

References
Explanation of Figures

All the photomicrographs were taken from sections of the cortex of each femoral diaphysis stained with hematoxylin and eosin. Figures 1 to 6 present periosteal osteogenesis and Figures 7 and 8 endosteal one.

Fig. 1. Cross section of the posterior part. Male 1 day old. Slight proliferation of the periosteum, which is rich in osteoblasts, and slight formation of osseous trabeculae are present around the embryonic bone. ×140.

Fig. 2. Cross section of the posterior part. Female 5 days old. Active periosteal osteogenesis is shown around the embryonic bone (downward). It consists of proliferation of the periosteum with many osteoblasts and formation of osseous trabeculae and canal systems (primitive haversian systems). The canal systems contain blood vessels and osteoblastic layers. ×115.

Fig. 3. Longitudinal section of the anterior part (compare with the posterior part illustrated in Fig. 4). Male 20 days old. It shows formation of canal systems which are fairly uniform and slant obliquely, and some of which anastomosing with one another. ×86.

Fig. 4. Longitudinal section of the posterior part. Male 20 days old. It shows essentially the same changes as those in Fig. 3, except that both canal systems and osseous trabeculae are more irregular-shaped. Anastomosing feature is evident among the canal systems. This cortex was about three times as thick as the opposite one. ×57.

Fig. 5. Cross section of the posterior part. Male 45 days old. Apposition by new bone tissue (upper half) is in progress around the embryonic bone (lower half). Osteoid tissue is apparent beneath osteoblastic layers lining newly formed canal systems. There is slight proliferation in the periosteum. ×115.

Fig. 6. Cross section of the posterior part. Female 90 days old. Bone tissue is close to maturity with outer basic lamella formed (right side). Osteoid tissue is still present beneath the periosteum. ×115.

Fig. 7. Cross section of the posterior part. Male 5 days old. Osteoid tissue is present beneath the endosteum rich in osteoblasts which line the inner surface of the medullary cavity. ×115.

Fig. 8. Cross section of the posterior part. Female 80 days old. Bone tissue is close to maturity with inner basic lamella formed. ×115.