

## A study of the normal development of the leg skeleton in the quail (*Coturnix coturnix japonica*)

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### INTRODUCTION

The avian leg skeleton has not departed as far from the primitive pentadactyl limb condition as has the wing skeleton and consequently it has provoked considerably less interest among anatomists. Probably for this reason the amount of information regarding the normal development of the leg skeleton is remarkably scarce. A noteworthy exception is a study by Johnson (1883) into the morphogenesis of the leg of the chick, but in this account the early stages of skeletal development are described in detail, and the later stages are ignored.

Most investigations relating to the embryonic development of the avian leg refer to the chick, with a few exceptions [Zehnter (1890) in the zebra parakeet; Broom (1906) in the ostrich; Sieglbauer (1911) in the duck; Holmgren (1933) in *Larus*]. Several studies in the chick deal with particular aspects of leg development, such as the development of the knee joint (Kaczander, 1886; Niven, 1933; O'Rahilly & Gardener, 1956) or the development of one particular bone, e.g. the tibiotarsus or tarsometatarsus (Fell, 1925; Schryver, 1967), with only casual references being made to other parts of the limb skeleton. The appearance of various centres of ossification in the leg skeleton has been the subject of other studies (Fujioka, 1955; Neumeister, 1966), but these accounts are also incomplete, because important elements such as the tarsal complex or the patella, which show minimal ossification in the embryonic period, were disregarded.

Despite the volume of experimental and anatomical information concerning the chick skeleton there is no really complete study on the morphogenesis of the leg skeleton, even in this species. In this study the normal development of the leg skeleton in the Japanese quail (*Coturnix coturnix japonica*) was examined from its initial appearance as mesenchymal condensations to the final stages of the embryonic period. This information was compared to the rather scattered data on the chick limb and the dissimilarities discussed.

### MATERIAL AND METHODS

Quail eggs were incubated in a Western Automatic incubator, at 99.7 °F and 60% R.H. and 10–20 embryos were collected daily from 5 to 16 d. The legs were excised and fixed in 10% neutral formalin for histological studies or in 95% ethyl alcohol for potash maceration and alizarin staining (Dawson, 1926).

For histological examination, the legs were processed and embedded in paraffin wax in the usual way. These tissues were serially sectioned at 5–7  $\mu\text{m}$  thickness and the sections were stained by haematoxylin and eosin, haematoxylin and Van Gieson counterstain or by a silver impregnation-toluidine blue method (Lansdown, 1968) to demonstrate sites of calcification and chondrogenesis.

Linear measurements of the skeletal elements were made from the alizarin stained material using a dissecting microscope ( $\times 20$ ,  $\times 60$ ). The tissue was illuminated by a phase contrast condenser to demonstrate the unstained cartilaginous tissue from the surrounding muscle. An estimate of the volumes of the cartilage, bone marrow and

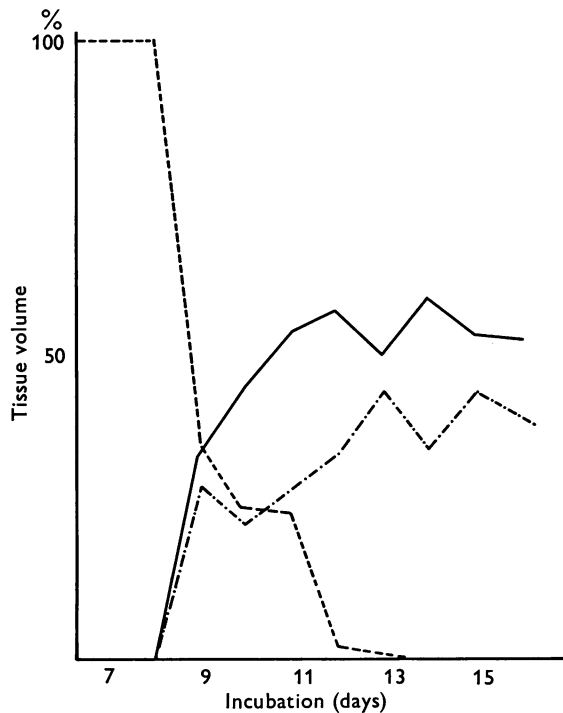


Fig. 1. The daily changes in the volume of cartilage, bone marrow and osseous tissue in the diaphysis of the quail femur during embryonic development. —, Osseous tissue; --- cartilage; - · - ·, bone marrow.

osseous tissue in the diaphysis of the femur was obtained from the histological material using an eye piece graticule and a point counting technique (Dunnill, Anderson & Whitehead, 1967). The technique was similar to that employed in a comparable study of the development of the embryonic wing skeleton (Lansdown, 1969) and the results were expressed in a similar manner.

## RESULTS

### *The histogenesis of the diaphysis of the femur (Fig. 1)*

At 5 d incubation no mesenchymal condensations are evident in the limb bud, but by 6 d not only are prominent condensations visible in both femoral and tibial

regions, but occasionally early cartilage formation is present also. However, even in the most advanced embryos at 6 d, the cartilage matrix is minimal in comparison to the cellular tissue, and discrete lacunae are lacking. The chondroitin sulphuric acid content of the cartilage matrix, as demonstrated by its metachromatic staining property with toluidine blue dye (Lansdown, 1968), is minimal.

By 7 d the cartilage matrix has increased considerably in volume and now occupies approximately 57% (determined using the graticule technique) of the total tissue mass. The intercellular distance is greatly increased and the cells are enclosed in discrete lacunae. The higher concentration of chondroitin sulphate in the walls of these

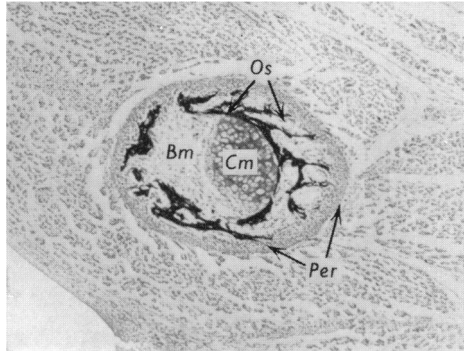


Fig. 2. Transverse section of the diaphysis of the femur of a 9 d quail embryo to demonstrate perichondral ossification (*Os*), degenerate cartilage (*Cm*) and bone marrow (*Bm*). (toluidine blue-silver impregnation method)  $\times 30$ .

lacunae in comparison to the intercellular areas greatly enhances their appearance. By 7 d incubation the diaphysis of the femur is ensheathed in a well differentiated fibrous perichondrium, which later in the seventh day assumes a bilaminar appearance. It now consists of an outer layer of fibrous tissue, and an inner zone of cells which resemble mesenchyme. On account of their morphology, their disposition and their subsequent association with collagenous fibres and granules of calcification, these cells are presumed to be osteoblasts. However, although a mildly positive alizarin staining is frequently present in potash macerated tissues (Dawson's test for calcification, 1926) no calcification can be demonstrated histologically until at least 9 d due to the very low concentrations present. At 8 d there is little obvious change in the histological appearance of the femoral diaphysis other than a definite increase in the perichondral osteogenic tissue and an initial hypertrophy of the chondrocytes in sub-perichondral areas.

The histological appearance of the femur at 9 d (Figs. 1, 2) is very different to that seen at 7 or 8 d. Osseous tissue and bone marrow are very prominent and the cartilage is in an advanced state of degeneration. This degeneration, which commenced during the eighth day, progresses rapidly until about 12 d, when the only cartilage remaining in the mid-shaft region of the femur is in the form of small islets of hypertrophic tissue. No cartilage is evident in these areas by 14 d and the endosteal cavity is occupied only by haematopoietic tissue.

Osseous tissue and bone marrow increase rapidly in volume from 8 to 12 or 13 d incubation, and by this stage they have similar proportions to those seen in the embryo at the prehatching stage, i.e. 55 and 40% respectively.

*Growth in cartilage and bone of the leg skeleton*

The skeletal elements of the leg skeleton may be considered in two series; a primary series comprising the femur, tibia and fibula (derived from the central mesenchyme of the early limb bud) and a secondary series consisting of the tarsal and metatarsal complexes and the phalanges (derived from the apical tissue of the early limb bud).

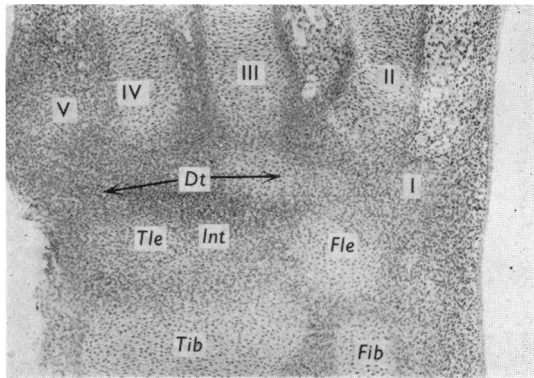


Fig. 3. Longitudinal section of the tarsal region of the 6-day quail embryo to demonstrate the fibula (*Fib*), tibia (*Tib*), tibiale (*Tle*), intermedium (*Int*), fibulare (*Fle*), distal tarsals (*Dt*) and 5 metatarsals (I, II, III, IV & V). (toluidine blue-silver impregnation method)  $\times 30$ .

In the quail embryo an early formation of elements of both series occurs at 5–6 d, and at 6 d early cartilage formation is present in all three primary elements and in the tarsal and metatarsal elements. A distinct proximo-distal gradient of maturity exists in all these centres with early cartilage present in the femur and tibia, and only mesenchymal condensations in the tarsal and metatarsal regions.

The fibula, which is considerably shorter than the tibia in the older embryo, is similar in length at 6 d, when it extends from the distal extremity of the femur to the tarsal region where a corresponding tarsal element is present. A tarsal element corresponding to the tibia appears to be composed of two elements at 6 d, which are in an advanced state of fusion (Fig. 3). In these early tarsal cartilages the peripheral tissues tend to be confluent and to merge into the surrounding mesenchyme. A similar condition exists at the knee joint, where the distal epiphysis of the femur, the presumptive tissue of the patella and the proximal extremities of the tibia and fibula are confluent. At the knee and ankle joints articular surfaces are not apparent until at least 13 or 14 d.

The tibia and fibula begin to ossify during the seventh day and the subsequent differentiation of the two bones is similar to the femur described above. However, cartilage formation and osteogenesis at the distal epiphysis of the fibula are arrested at 7–8 d and only fibrous perichondral tissues remain at later stages.

Chondrogenesis begins in the distal elements of the leg skeleton in the 6–7 d period and by 8 d incubation a complete series of tarsal, metatarsal and phalangeal cartilages is present, consisting of 6 tarsal elements, 5 metatarsals and 14 phalanges

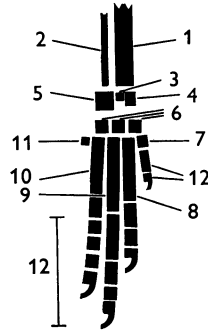


Fig. 4. A diagram of the elements identified in the tarsal, metatarsal and phalangeal region of the quail embryo. 1, Tibia; 2, Fibula; 3, Intermedium; 4, Tibiale; 5, Fibulare; 6, Distal tarsals; 7, Metatarsal I; 8, Metatarsal II; 9, Metatarsal III; 10, Metatarsal IV; 11, Metatarsal V; 12, Phalanges.

Table 1. Sequence of appearance of centres of ossification in the elements of the leg skeleton

Age (days)	Femur	Tibia	Fibula	Tibiale	Fibula	Inter-medium	Metatarsal			
							I	II	III	IV
7 d	+	+	+( $\frac{3}{4}$ )	—	—	—	—	+( $\frac{1}{4}$ )	+( $\frac{1}{4}$ )	—
8 d	+	+	+	—	—	—	—	+	+	+
9 d	+	+	+	—	—	—	—	+	+	+
10 d	+	+	+	—	—	—	—	+	+	+
11 d	+	+	+	—	—	—	—	+	+	+
12 d	+	+	+	—	—	—	+( $\frac{3}{6}$ )	+	+	+
13 d	+	+	+	—	—	+( $\frac{2}{7}$ )	+	+	+	+
14 d	+	+	+	—	—	+( $\frac{5}{7}$ )	+	+	+	+
15 d	+	+	+	—	—	+	+	+	+	+
16 d	+	+	+	+( $\frac{5}{6}$ )	+( $\frac{5}{6}$ )	+	+	+	+	+

Age (days)	Phalanges													
	I <sup>1</sup>	I <sup>2</sup>	II <sup>1</sup>	II <sup>2</sup>	II <sup>3</sup>	III <sup>1</sup>	III <sup>2</sup>	III <sup>3</sup>	III <sup>4</sup>	IV <sup>1</sup>	IV <sup>2</sup>	IV <sup>3</sup>	IV <sup>4</sup>	IV <sup>5</sup>
7	—	—	—	—	—	—	—	—	—	—	—	—	—	—
8	—	—	—	—	—	—	—	—	—	—	—	—	—	—
9	—	—	+( $\frac{4}{7}$ )	+( $\frac{3}{7}$ )	—	+( $\frac{6}{7}$ )	+( $\frac{2}{7}$ )	—	—	—	—	—	—	—
10	+( $\frac{1}{4}$ )	—	+	+( $\frac{3}{4}$ )	+( $\frac{1}{4}$ )	+	+	+( $\frac{1}{4}$ )	+( $\frac{1}{4}$ )	+	—	—	—	+( $\frac{1}{4}$ )
11	+	+( $\frac{1}{7}$ )	+	+	+( $\frac{6}{7}$ )	+	+	+	+	+	+( $\frac{4}{7}$ )	+( $\frac{1}{7}$ )	—	+( $\frac{4}{7}$ )
12	+	+( $\frac{5}{6}$ )	+	+	+	+	+	+	+	+	+	+( $\frac{5}{6}$ )	+( $\frac{3}{6}$ )	+( $\frac{5}{6}$ )
13	+	+	+	+	+	+	+	+	+	+	+	+	+	+
14	+	+	+	+	+	+	+	+	+	+	+	+	+	+
15	+	+	+	+	+	+	+	+	+	+	+	+	+	+
16	+	+	+	+	+	+	+	+	+	+	+	+	+	+

The normal sequence of appearance of centres of ossification in the various elements of the embryonic leg of the quail (in some cases the number of embryos showing ossification is given as a fraction of those examined, i.e.  $\frac{4}{7}$ ).

(Fig. 4). Metatarsal V at 8 d is in a state of degeneration and at 9 or 10 d no evidence of this element is visible. Ossification is seen in metatarsals II, III and IV at 8 d and in their proximal phalanges at 9 or 10 d (Table 1). Some irregularities occur however in the order of ossification in the elements of the secondary skeleton. For example, the bones of the tarsal region do not ossify until considerably later than more distal elements, e.g. metatarsals or phalanges. Although ossification in the metatarsal region (II, III and IV) commences at 7 to 8 d, metacarpal I is not seen to ossify until 12 d. This retardation of ossification might be due to the rotation of this digit from the lateral position seen at 6 to 7 d (Fig. 3) to the anisodactylous condition seen at 9 d. The phalanges of digit I ossify before the corresponding metatarsal, at days 10 or 11 of incubation, and thus only slightly later than the phalanges of digits II, III and IV and are thus little affected by the rotation of the digit. Another irregularity in the ossification of the phalanges is in digit IV where the terminal element (IV<sup>5</sup>) ossifies prior to the three more proximal digits (IV<sup>2</sup>, IV<sup>3</sup> and IV<sup>4</sup>).

Later in the embryonic period centres of ossification appear in the tarsal region (Table 1). At 13 d incubation a focus of ossification is seen in the vicinity of the distal epiphysis of the tibia. Histologically this element can be identified as an ascending process of the intermedium which extends proximally in a groove in the anterior face of the tibia. Two other centres of ossification appear in the tarsal region at 16 d incubation, and correspond to the fibulare and tibiale.

#### *Tarsal elements*

The six elements which are present in the tarsal region at 6–7 d form a proximal and distal series. In the proximal row the element lying opposite the fibula is presumably the fibulare and two almost completely fused elements which correspond to the tibia are probably the intermedium and tibiale. At 7 d fusion has also occurred in the three elements of the distal series and by 9–10 d the tarsometatarsus is formed by a fusion of this distal tarsal complex to the proximal extremities of metatarsals II, III and IV. The elements of the proximal series are fused together by 11 d, and by 12 d they are fused to the epiphysis of the tibia and the tibiotarsus is formed. This union is strengthened considerably by an ascending process of the intermedium which extends proximally in a groove in the anterior face of the tibia, and which becomes fused to it later in hyaline cartilage. Therefore at 12 d no separate elements remain in the tarsal region.

#### *Patella*

The patella in the adult bird is a small sesamoid bone in the anterior aspect of the knee joint, associated with the quadriceps muscle. Embryologically it appears at 8–9 d incubation as a mesenchymal condensation. Ossification does not occur until several weeks after hatching, and for most of the embryonic period the patella is poorly developed in cartilage. At 11 d two zones are identifiable in this cartilage, one adjacent to the femur in which the cartilage is better developed, and a distal zone. This zoning is lost by 13–14 d but the cartilage is far from mature, in fact no perichondrium is apparent until at least 15 or 16 d.

*Bone growth and changes in the proportions of the skeletal segments during embryonic growth*

The linear growth rate of the three skeletal segments, i.e. femur, tibia and tarsal/metatarsal/phalanges, measured as the percentage daily increase (Fig. 5) rises to a peak at 7–8 d which corresponds to a period of high growth in cartilage, and to two secondary peaks at 12 and 14–15 d which can be related to a high rate of osteogenesis seen histologically (Fig. 1). Furthermore the rates of growth in the individual segments are similar to that exhibited by the leg skeleton as a whole.

The proportion of a segment to the entire limb changes as the limb grows. For example, at 7 d the femur forms approximately 30% of the leg length (Fig. 6), yet



Fig. 5. The percentage daily increase in the length of the femur, tibia and tarsal/metatarsal phalanges segments. The percentage daily increase is calculated as

$$\frac{\text{length on day 1} - \text{length on day 0}}{\text{length on day 0}} \times 50.$$

—, Femur; ---, tibia; - - -, tarsal/metatarsal/phalanges.

due to the higher rate of growth in the two distal segments, the percentage declines such that at 16 d it forms only 25%. In contrast, the distal segment which shows the highest growth rate increases in proportion from 38% at 7 d to 43% at 16 d.

At 7 d incubation an early zone of calcification is seen ensheathing approximately 27% of the total length of the femur and tibia. Throughout the remainder of the embryonic period as well as into the post-embryonic period these ossification zones

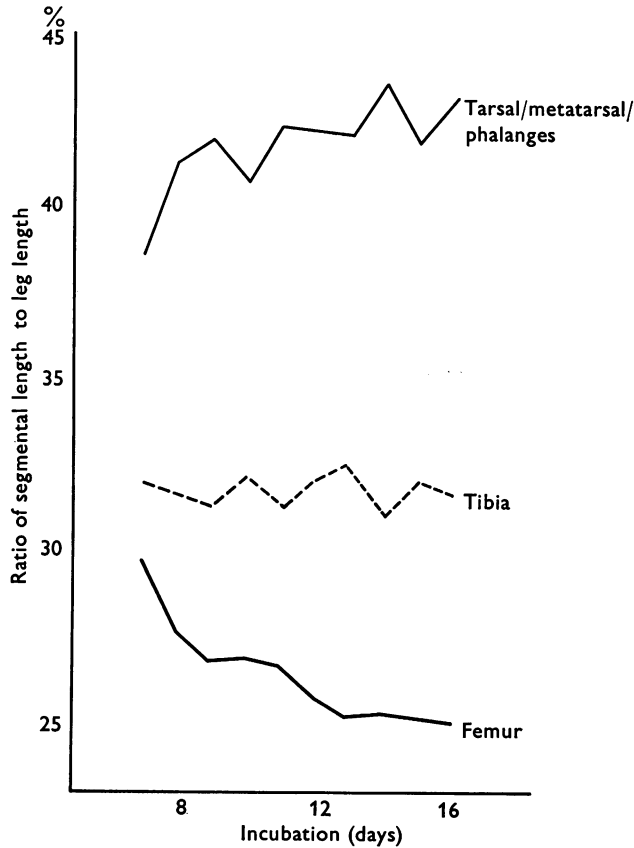


Fig. 6. The daily changes in length of the femur, tibia, and tarsal/metatarsal/phalanges segments as a percentage of the total limb length.

increase in length, such that at 16 d they form 84 and 87% respectively (Fig. 7). The increase in the ratio of the bony diaphysis to the whole element length is considered to be indicative of the rate of ossification of the particular skeletal element. The increase in this ratio is high from 7–10 d incubation for the femur, tibia and fibula, lower from 10–11 d and high again from 11–14 or 15 d.

By hatching, therefore, the three elements of the primary limb skeleton are well ossified, and the bony shafts are robust and strong enough to support the quail chick in the early neonatal period.



## DISCUSSION

Fujioka (1955) suggested that the appearance of centres of ossification in the chick embryo might be used to determine the stage of development. A stage plan of this type was actually constructed for ageing tern embryos (Leighton, 1894), and for the quail embryo based on the development of the wing skeleton (Lansdown, 1969). It is possible to construct a similar table for the quail using the data on the normal development of the leg skeleton (Table 2).

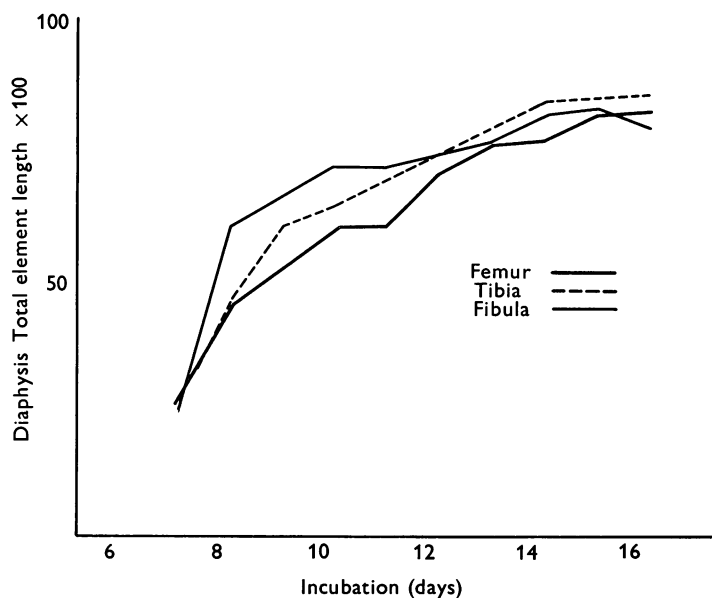


Fig. 7. The daily increase in the ratio of the bony diaphysis to the total length of the element.

*A comparison between the development of the leg skeleton in the quail and the domestic fowl*

Information relating to the normal development of the leg skeleton in the chick is widespread. Although variations occur in the times given for the initial mesenchymal condensation, cartilage formation and ossification in the major elements, it is quite clearly seen that the development of the limb skeleton in the quail and chick is very similar.

Mesenchymal condensation and early stages of cartilage formation are reported in the diaphysis of the chick femur, tibia and fibula at 5 to 6 d of incubation, and at 6 d well formed cartilage is seen in these elements (Johnson, 1883; Fell & Canti, 1934; Muratori & Franceschini, 1945; O'Rahilly & Gardener, 1956). Centres of ossification were also seen at this stage (Fell & Canti, 1934; O'Rahilly & Gardener, 1956), although Fujioka (1955) and Neumeister (1966) reported that the first centres of ossification to be seen were in the tibiae at 7–8 d. According to these workers, the

Table 2. *Stages in the development of the leg skeleton as a guide to embryonic ageing*

Stage	Age of embryo (days)	Histology of femur diaphysis	Development in cartilage of whole leg skeleton	Appearance centres of ossification	Linear growth rate	Length of diaphysis in femur and tibia	Fibula
I	1-5	Early cartilage	No skeletal development in any bones of the leg skeleton	—	} Equal in length in secondary skeleton	}	Tibia = fibula in length
II	6-7	Early cartilage	Chondrogenesis in femur, tibia and fibula; mesenchymal condensation in tarsal, metatarsals	—			
III	7-9	Mature cartilage and early ossification	Chondrogenesis in tarsals and metatarsals; mesenchymal condensation in phalanges	Femur, tibia; fibula metatarsals	High growth rate in all segments	20-50%	Fibula does not extend to tarsal region
IV	9-11	Calcification seen histologically; bone-marrow chondrolysis	Chondrogenesis in distal phalanges	Ossification in phalanges	Low growth rate in all segments	Over 50%	—
V	12-14	Little cartilage remaining; trabeculate bone	—	—	High growth rate in all segments	—	—
VI	14-16	Trabeculate bone and prominent bone marrow, never any cartilage	—	Ossification of three tarsal elements; intermedium, tibiale, fibula	General decline in growth rate	—	—

femur does not ossify until 8–9 d, and the fibula later still at 10–11 d. Centres of ossification in the quail seem to appear in the femur, fibula and tibia at approximately the same time. In the chick femur the excavation of the bone marrow cavity begins at 8 d incubation (O’Rahilly & Gardener, 1956), which is slightly earlier than in the quail.

In the chick the femur has been described as the stoutest element in the primary leg skeleton during the period of chondrogenesis, although its growth rate is inferior to that of the tibia (Stepanova, 1926). In the quail, however, not only is the tibia the stoutest element but its growth rate is higher, even in the cartilaginous stage. Furthermore, the chick fibula is reputed to have a more advanced rate of chondrogenesis than the tibia (Fell & Canti, 1934), yet in the quail at 6 days incubation the fibula is inferior in girth to the tibia, and at 7 d the ossification is less advanced. In addition, chondrogenesis and osteogenesis cease at the distal extremity as early as 7 d incubation, leaving only a single zone of growth, at the proximal epiphysis. Since O’Rahilly & Gardener (1956) mentioned that the fibula is considerably narrower than the tibia in transverse section, and that it tapers distally with ossification occurring mainly in the proximal extremity, it seems inconceivable that the fibula in the chick can ever have a higher rate of growth than the tibia.

In the tarsal region of the chick Johnson (1883) identified three cartilaginous elements, which appear in the 6 d embryo. Two elements form a proximal row, which later becomes fused to the distal extremity of the tibia and the third is a distal element which ultimately fuses with the metatarsal elements. Morse (1874) identified a third cartilage in the proximal tarsal series and he noticed that this element, which he called the intermedium, had an ascending process which grew up between the tibia and fibula and which later ossified. This centre of ossification has been missed in more recent investigations (Fujioka, 1955; Neumeister, 1966). Neumeister however reported a distinct centre between the tibia and the tarsometatarsus at 19 d incubation. This centre presumably corresponds to one of the three centres of ossification seen in the tarsal region in the quail.

All five metatarsals of the pentadactyl limb were seen in the chick at 6 d, in early cartilage (Johnson, 1883). At this stage metatarsal III is already noticeably longer than the remaining four, and metatarsal V the most insignificant. In later stages this latter element is not seen and is supposed to be fused to the basal tarsals (Lutz, 1942). Johnson (1883) further reported that metatarsal I split away from metatarsal II at 6–7 d and came to lie back. In most respects the metatarsal development in the quail is similar to that seen in the chick except that rotation of metatarsal I to a backward position occurs slightly later in the quail.

The phalangeal region in the chick has been little studied but Romanoff (1960) referred to the separation of precartilaginous tissue at the distal extremities of the metatarsals at 8 d. Fujioka (1955) mentioned that the centres of ossification in the phalanges of digits I, II and III appear in a proximo-distal sequence, but that in digit IV the phalanges ossify in the order 1, 4, 5, 2 and 3. A similar order of ossification is seen in the phalanges of the quail, although in digit IV the order of ossification seen in the quail is 1, 5, 2, 3 and 4.

The patella appears slightly earlier in the chick than in the quail at 6.5–7.5 d incubation (Renda, 1968), but like the quail the chick patella does not ossify until

after hatching, and until late in the incubation period it is present only as a poorly differentiated cartilage (O'Rahilly & Gardener, 1956; Renda, 1968).

From a comparison of the leg development in the quail and the chick, it is clear that the skeletal elements in both birds develop in a very similar sequence, even though the development in the chick is earlier than in the quail. The ultimate form of the limb in the two birds is very similar. Therefore, on the basis of these observations on the development of the hind limb, the chick and quail seem to be correctly classified in the order Galliformes (Wetmore, 1951).

Evolutionary pathways have frequently been revealed in the course of normal embryonic studies. One such study concerns the formation of the avian foot (Holmgren, 1933). In the embryo of *Larus* a single element is seen, distal to the tibia in which no fused cartilages can be identified. In *Ploceus*, however, such a differentiation is visible and two definite elements can be identified, a fibulare and a tibiale. In *Pygosceles*, Sieglbauer (1911) identified a third centre which is the intermedium; Holmgren (1933) believed that this composite cartilage consists of an intermedium and two centralia (1 and 2), and that the tibiale is missing. The fibulare at an early stage in *Pygosceles* is seen to consist of two fused elements, which Holmgren considered to be the fibulare and centrale 4. Centrale 3 is supposed to disappear during ontogeny. Holmgren (1933) does not refer to the fate of the distal tarsals or the metatarsals.

The form of the leg skeleton is typical of that seen in the majority of the species of birds, and a study of its normal development throws some light on the origin of birds from the primitive pseudosuchians. In some of these forms, as an adaptation to cursorial, bipedal locomotion, the elements of the lower leg achieved a greater length than the proximal element and the tarsus became specialized, in that the proximal elements were tightly bound to the crus (tibia and fibula). This trend was continued in the saurischian dinosaurs, the ligamentous attachment between the tibia and proximal tarsals being replaced by an ascending process on the astragalus (for a long time it has been known that this element in reptiles is derived from a fusion of the intermedium, tibiale and one of the centralia) which embraced the face of the tibia. In birds, derived along a separate line of pseudosuchian evolution, the requirements imposed by the cursorial habit necessitated a similar mechanism and the formation of the tibiotarsus (fusion of the proximal row of tarsals to the distal extremity of the tibia) is seen. The tibiotarsus is a feature of the skeleton unique to the avian class.

#### SUMMARY

Differentiation in the leg skeleton of the quail commences at 5–6 d incubation and by 6 d early cartilage is present in the diaphyses of the femur, tibia and fibula. An initial ossification of these elements occurs at 7–8 d and by 11 d most elements of the leg skeleton are ossifying. Centres of ossification occur in the tibiale, intermedium and fibulare at 13–16 d, when the elements are fused in cartilage to the tibia, and the tibiotarsus is formed. Three distal tarsals do not ossify individually but fuse to the metatarsals to form the tarsometatarsus.

Histologically the femur passes from cartilaginous to osseous states at 8–9 d, during which time there is a fall in the linear growth rate. Peaks of maximum growth

occur in the femur, tibia and tarsus/metatarsal/phalanges respectively at 7–8, 12 and 14–15 d and can be related to periods of high growth in cartilage and bone.

During embryonic development the tibia maintains a near constant proportion in length to the entire limb skeleton, whereas the femur declines in proportion and the distal segment gradually increases. Thus it can be seen that, in the embryonic period at least, this distal segment grows more rapidly than either the tibial or femoral segments.

The growth of the leg skeleton of the quail seems to be very similar to that seen in the chick embryo.

From the observations it is possible to construct a staging plan for ageing the embryo which outlines criteria for six stages of growth based on the growth and differentiation of the limb skeleton.

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