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An investigation of the development of the wing skeleton in the quail (*Coturnix c. japonica*)

A. B. G. LANSDOWN

British Industrial Biological Research Association, Woodmansterne Road, Carshalton, Surrey,
and
*Birkbeck College, University of London, Malet Street, London, W.C.*1.

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INTRODUCTION

The anatomy of the avian wing skeleton and its patterns of differentiation have been extensively examined (Parker, 1868, 1888; Knopfli, 1919) and attempts have been made to analyse the factors involved in the control of its differentiation (Saunders, 1948; Amprino & Camosso, 1958; Amprino & Amprino Bonetti, 1967) and to establish the evolutionary pathway from the pentadactyl limb (Montagna, 1945). Most of this work, with few exceptions (Leighton, 1894; Seiglbaur, 1911; Maillard, 1948), relates to the common fowl (*Gallus domesticus*). Little information is available on the embryology of the wing skeleton in the quail (*Coturnix c. japonica*), which is rapidly becoming a popular bird in many fields of research, including toxicology and teratology.

Because of the wealth of information available on the development of the common fowl, it was considered important to compare the results of this study on the quail wing with available data on the former species. Such a comparison is considered of taxonomic significance since the two species have been classified in different sub-orders of the Galliformes (Wetmore, 1951).

The development of the long-bones in the fowl has been described in detail (Fell, 1925). The histogenic process involved in the development of a long-bone (which includes all major elements of the wing skeleton) is a differentiation of mesenchyme cells into chondroblasts and osteoblasts. These cells are identified histologically by their disposition, morphology and the nature of their matrix. In this study the differentiation of mesenchymal cells into chondroblasts and osteoblasts and the mode of development of cartilage and osseous tissue in the humerus is described in detail with particular reference to the age of the embryo. Reference is also made to the differentiation of other elements of the wing.

MATERIALS AND METHODS

Eggs used in this study were of the Beecham Research Laboratory strain of the Japanese quail. They were incubated in a Western Automatic incubator, after the procedure of Padget & Ivey (1959), and harvested daily from 6–16 d (quail chicks hatch at 16·5 d). Incubation was timed from approximately 2 h after the eggs had

been placed in the incubator, in order to allow them sufficient time to attain the temperature of the incubator. Freshly laid eggs (less than 12 h old) were used in this work in order to eliminate the reduced hatchability risk which occurs with stored eggs.

Embryos removed from the shell were freed of their membranes and blotted to remove any amniotic fluid or adhering yolk and then transferred to a glass dish.

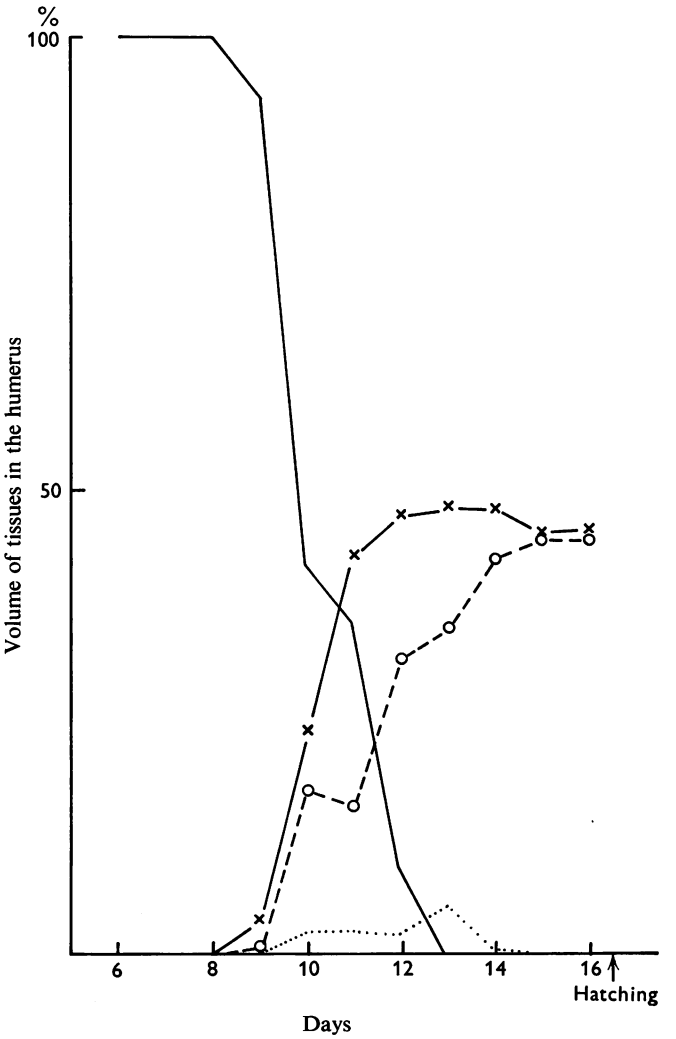

Fig. 1. The daily changes in proportion of cartilage, chondrolytic tissue, osseous tissue and bone-marrow present in the diaphysis of the humerus of the quail during embryonic development. —, Cartilage; ····, chondrolytic tissue; ×—×, osseous tissue; O--O, bone marrow.

They were fixed in either neutral 10% formalin for histological studies or in 95% ethyl alcohol for potash maceration and alizarin staining (Dawson, 1926).

Wings were excised from embryos after 96 h fixation for histological examination. They were thoroughly washed free from fixative before processing and embedding in

paraffin wax in the usual way. Serial sections of 5–7 μm thickness were cut across the entire wing. These sections were subsequently stained by haematoxylin and eosin to show general cytological detail, haematoxylin and Van Giesen counterstain to demonstrate collagen and by a silver impregnation–toluidine blue method (Lansdown, 1968) to demonstrate sites of calcification and chondrogenesis.

An integrating eyepiece containing a graticule of 100 points was used to obtain quantitative data of the histological sections (Dunnill, Anderson & Whitehead, 1967). The central sections of the mid-diaphysis region of the humerus were surveyed and 1000–2000 point counts were made. The number of points lying on any one tissue component (e.g. cartilage, calcified tissue, bone marrow) is proportional to the area of that tissue in the section (Dunnill, 1962) and is also proportional to its volume (Delesse, 1848). Counts were made at a magnification of $\times 180$ for young embryos (7–10 d incubation) and $\times 72$ and $\times 30$ for older embryos. Several embryos were used in any one age group and several incubation batches were employed. The data were averaged and expressed graphically (Fig. 1).

Table 1. *The normal sequence of appearance of centres of ossification in the various elements of the embryonic wing of the quail*

(In some cases the number of embryos showing ossification is given as a fraction of those examined, i.e. $\frac{1}{8}$.)

Age in days	H	U	R	McIII	McIV	PII^1	PII^2	PIII^1	PIII^2	PIV^1
7	+	+	+	+ ($\frac{1}{8}$)	+ ($\frac{1}{8}$)	−	−	−	−	−
8	+	+	+	+	+	−	−	−	−	−
9	+	+	+	+	+	−	−	−	+ ($\frac{2}{3}$)	−
10	+	+	+	+	+	+	−	+	+	−
11	+	+	+	+	+	+	−	+	+	−
12	+	+	+	+	+	+	+ ($\frac{2}{4}$)	+	+	−
13	+	+	+	+	+	+	+	+	+	−
14	+	+	+	+	+	+	+	+	+	−
15	+	+	+	+	+	+	+	+	+	+ ($\frac{1}{8}$)
16	+	+	+	+	+	+	+	+	+	+

H, Humerus, U, ulna, R, radius, Mc, metacarpal, P, phalanx.

Linear measurements were made from embryos prepared by the potassium hydroxide maceration–alizarin staining technique. The lengths measured were those of the total length of primary elements—humerus, radius and ulna, as well as the total length of the manus (carpals, metacarpals and phalanges). This latter measurement was the distance from the distal end of the ulna to the distal extremity of the terminal phalanx of digit III. In addition the lengths of the regions of perichondral ossification in the diaphyses of the primary elements were measured. Measurements of wing elements of 7–10 d embryos were obtained using an eyepiece graticule with the skeletal elements illuminated by a phase-contrast condenser. By this means cartilage could be clearly demonstrated in contrast to the surrounding muscle. In the case of embryos of 7 or 8 d incubation, where the epiphyses were indistinct, the distance was obtained across the greatest length of identifiable cartilage. Centres of ossification were generally easily measured since the alizarin-stained ossified tissue

contrasted well against the potash-cleared surrounding tissue (muscle and cartilage). Since skeletal elements of the older embryos (10–16 d incubation) were considerably larger than those of 6–9 d embryos, measurements were possible with accuracy under a dissecting microscope. Wings were prepared rather differently for this mensuration. The muscle layer was dissected away to expose the skeletal elements and accurate measurements of the diaphyses were made before the bones were treated with a few drops of 0·5 % aqueous toluidine blue. This coloured the cartilaginous epiphyses blue and made possible an accurate measurement of the total bone length.

The daily increases in length of the skeletal segments (humerus, radius/ulna, manus) were investigated and observations were made of changes in their relative proportion and this was related to the histological pattern of the various bones. Such a correlation between histogenesis and linear dimensions has been absent in previous studies.

The alizarin preparations were used to demonstrate the sequence of appearance of ossification, particularly in the cartilages of the manus (Table 1).

RESULTS

(*a*) *The sequence of histogenesis in the diaphysis of the humerus* (*Fig.* 1)

Cartilage is first identified in the diaphysis of the humerus at 6 d incubation. Its immaturity is indicated by the lack of well-defined lacunae and the low metachromasia in the intercellular matrix (the metachromasia with toluidine blue dye is a measure of the amount of chondroitin sulphate present). The cellular material consisting of chondroblasts and chondrocytes occupies approximately 44 % of the total volume at this stage, the only other tissue component present being the intercellular matrix. In the diaphysis of the 6–7 d embryo the cartilage becomes considerably more mature and by 7 d well-defined lacunae and increased metachromasia in the intercellular matrix can be seen. Peripheral to the cartilage a clear bilaminate perichondrium is present, in the inner or osteogenic layer of which early osteogenic activity can be seen. Osteoblasts, which appear as cells having irregular shapes, are seen peripheral to the cartilagenous 'core' (Fig. 2). Chondrolytic activity is not seen however until 8–9 d of incubation. Histologically no calcification is demonstrable due to the very low concentration present, but evidence for calcification is seen in the alizarin stained preparations, this test being more senstive. Calcified tissue is readily seen in humerus sections prepared from 8 and 9 d embryos.

At 8–9 d incubation a transformation of the mid-diaphysis from the chondrous to osseous phase commences. Over the 8–13 d period there is a precipitous decline in the amount of cartilage present, with a corresponding increase in osseous tissue. Associated with the degeneration of the cartilage phase is the appearance of invading blood capillaries and a formation of haematopoietic tissue. This tissue increases in volume from 8 d through to hatching, at which time it occupies a similar volume to that of the osseous tissue, that is approximately 45 %. The chondrolysis which has commenced in the 8–9 d period of incubation is not complete in the diaphysis until 15 d, although after 12 d the only cartilage seen is in the form of small islands of hypertrophic tissue.

The proportion of calcified tissue present in the mid-diaphysis at 12 d is very

similar to that seen in the embryo immediately prior to hatching. In the 12–16 d period there is a gradual increase in the amount of haematopoietic tissue, which replaces the degenerating cartilage (Fig. 1).

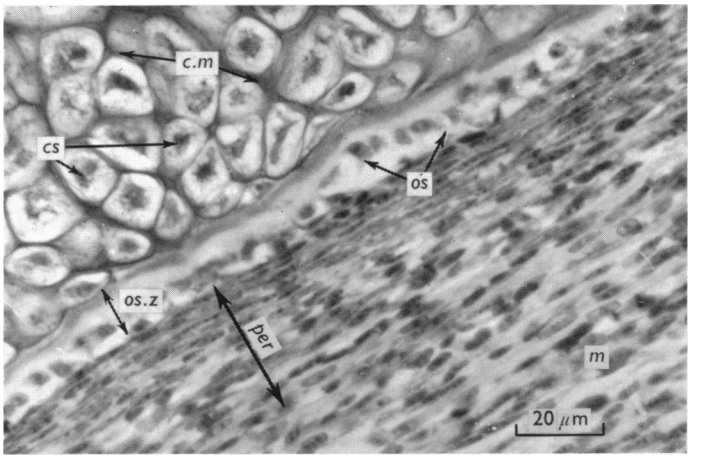

Fig. 2. Longitudinal section across the diaphysis of the developing humerus of the 7 d quail embryo to show the bilaminate perichondrium (*per*) and the initial appearance of osteoblasts (*os*) in the inner osteogenic zone (*os.z*) of the perichondrium. (*m*, undifferentiated mesenchymal tissue; *c.m*, cartilage matrix; *cs*, chondrocytes).

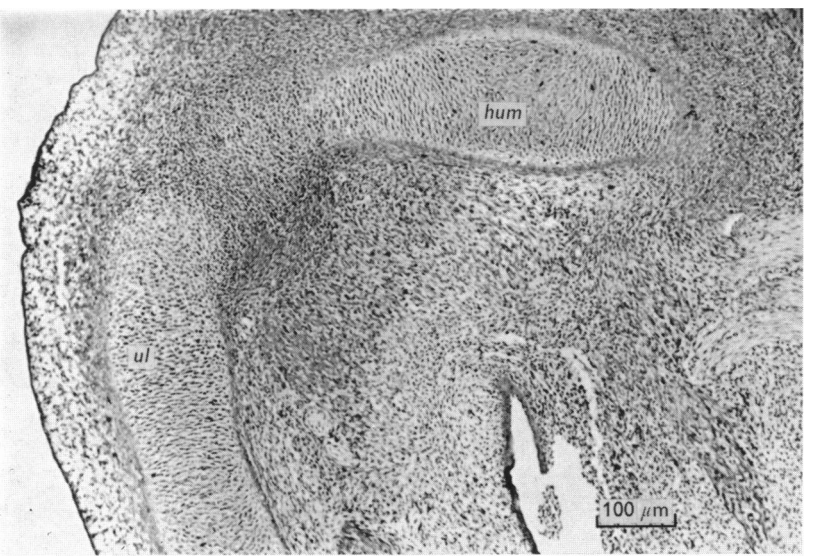

Fig. 3. Longitudinal section across the wing of the 6 d quail embryo to demonstrate early cartilage formation in the diaphyses of the humerus (*hum*) and ulna (*ul*).

(b) Growth in cartilage and bone of the wing skeleton

The wing skeleton consists of two series of bones, the primary series comprising the humerus, radius and ulna which arise from the mesenchyme of the wing bud, and the secondary series which includes the carpal, metacarpal and phalangeal elements which are derived from the apical cap of the wing bud (Saunders, 1948). In the quail the elements of the primary series appear in the 6 d embryo (Fig. 3) and develop in a similar manner to that of the humerus, referred to above. The ossification which appears in all these elements at approximately the same time proceeds in a very comparable fashion (Table 2) until 14 d when the proportion of the diaphysis to the total bone length in the radius and ulna is greater than that in the humerus. In the secondary series chondrogenesis and osteogenesis in the various bones appear in a proximo-distal sequence, except that the metacarpals III and IV appear in advance of the carpal elements. The sequence of chondrogenesis in this region of the wing skeleton is, first, an appearance of centres of chondrogenesis for metacarpals III and IV at 7 d, followed by centres for the three proximal carpals, the three distal carpals and the proximal phalangeal elements of digits II, III and IV at 8 d. Finally at 9 d further phalanges in digits II and III appear and also metacarpal II. The sequence of ossification follows a similar order, except for some irregularities seen in Table 1 (i.e. the appearance of ossification in the distal phalanx of digit III in advance of the proximal element, and a delayed ossification in the phalanx of digit IV).

Table 2. *The daily change in length of the diaphysis as a percentage of the total bone length in the humerus, ulna and radius.*

Age	Humerus	Ulna	Radius
7	29·08	29·65	30·20
8	56·19	55·14	52·04
9	51·87	53·54	52·92
10	60·26	68·68	66·67
12	69·59	69·74	67·44
14	68·64	70·01	68·44
16	70·26	76·73	76·82

(c) Carpal elements

Only six carpal elements can be identified in the wing in the quail. Of these, three form a proximal series: two precartilages in process of fusion opposite the distal extremity of the radius, which may be homologous to the radiale and intermedium of the pentadactyl limb as supposed by Montagna (1945), and a third element which is adjacent to the distal epiphysis of the ulna, presumably corresponding to the ulnare. No evidence is apparent for elements of the centralia series. A distal series of three carpals is present. These elements are seen in a precartilaginous form and are in the process of fusion to form a crescentic element which appears in 8 or 9 d embryos as a cartilage close to the proximal extremities of the metacarpal complex and which ultimately fuses with it. No ossification is seen in the carpal region in the embryonic period.

(*d*) *Bone growth and changes in proportion of the segments of the wing during the embryonic period*

The growth rate of the three segments of the wing, humerus, ulna or manus was measured in each case as the percentage daily increase in bone length (Fig. 4). The three segments show a very clear plan of biphasic growth, with peaks at 8–9 and 12 d. These peaks correspond to periods of maximum activity of the chondrogenic and osteogenic phases. The decline in the growth rate of all three segments at 10 d presumably represents the final stage of transition from cartilaginous to osseous

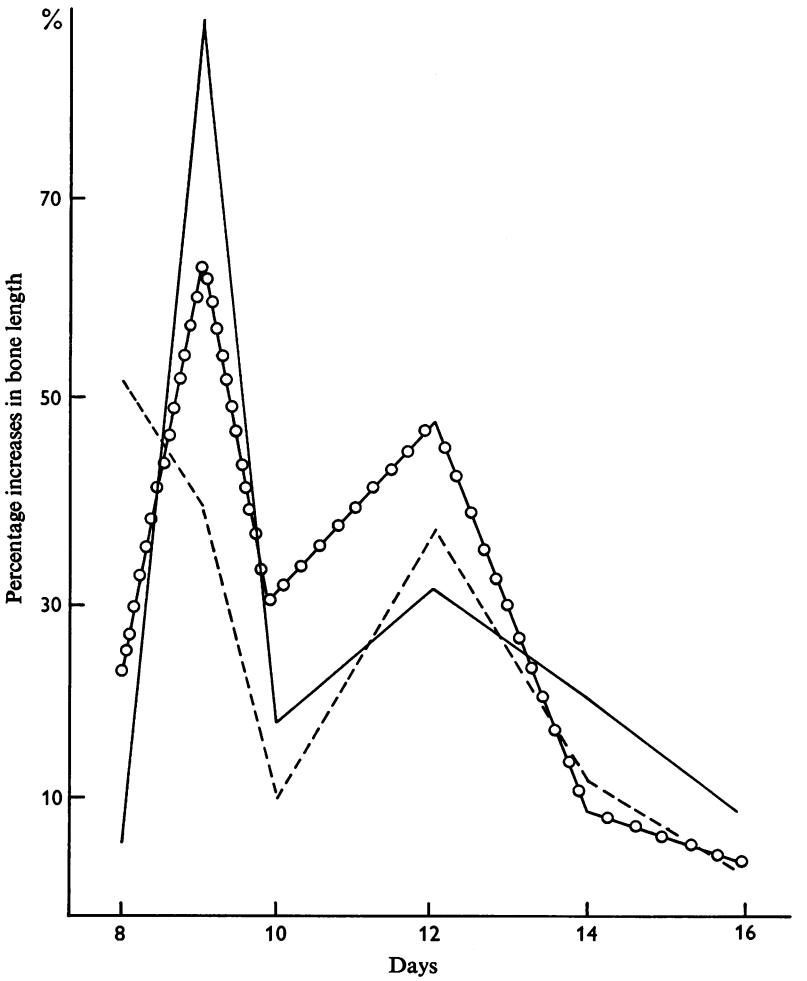

Fig. 4. The daily percentage increase in the length of the humerus, ulna and manus (carpal+metacarpal+phalanges). The percentage daily increase is measured as

$$\frac{\text{length on day 2—length on day 1}}{\text{length on day 1}} \times 100.$$

–, Humerus; —○—○, ulna; - - -, manus

states. Since the major skeletal elements of the wing have developed to near-hatching size by 12 d there is a consequent decrease in growth rate from this time until hatching.

Since growth is not equal at all stages in all segments, there is consequently a change in proportion of one part to another. In Fig. 5 it is seen that at 7 d the length of the humerus forms 38 % of that of the lower wing (ulna + manus) and 27 % of the total wing, but with the commencement of growth in the manus on the seventh day both proportions show a decline to 29 and 22 % respectively with a subsequent recovery by 10 d to approximately the 7 d proportions, after which time there is only slight change. The growth rate of the humerus at hatching is higher than either of the lower segments (Fig. 4). It may be concluded that this growth rate will be maintained into the post-hatching period until the proportion seen in the adult bird is attained.

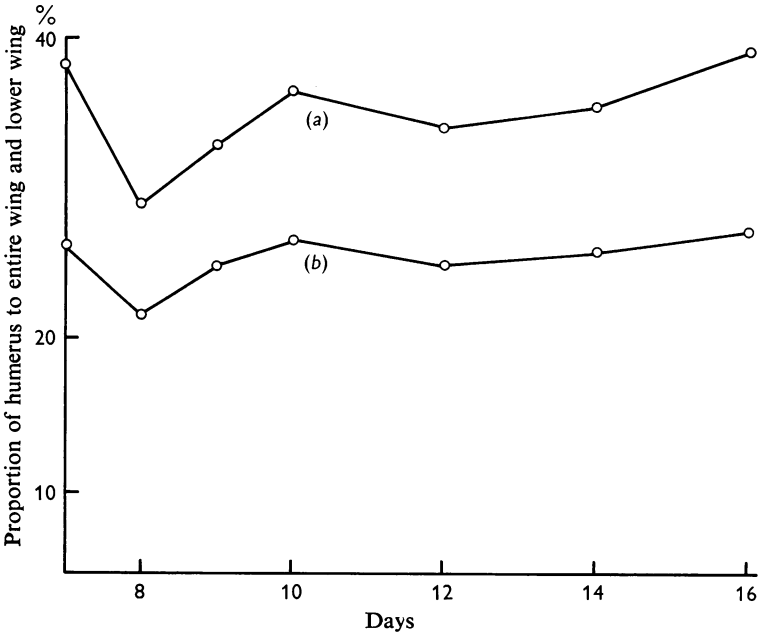

Fig. 5. The daily change in proportion of the humerus to (*a*) the length of the lower wing (ulna + manus) ([humerus/lower wing] × 100); (*b*) the length of the entire wing skeleton ([humerus × entire wing] × 100).

DISCUSSION

Leighton (1894) suggested a stage-by-stage sequence for the development of the wing skeleton in the tern (*Sterna wilsoni*) based mainly on observations made on alizarin-stained material. A similar plan can be constructed, from the data given in this study, for the development of the wing skeleton in the quail (Table 3).

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

Montagna (1945) dealt very thoroughly with the development of the wing in the domestic fowl. A comparison of his observations with those of the present study leads to the conclusion that the chick wing skeleton begins to develop slightly earlier than that in the quail. For example, whereas immature cartilage is not seen in the diaphyses of elements of the primary wing of the quail until 6 d, it is seen in chick as early as 4–5 d. This cartilage is mature in the chick by 6 d. Ossification is apparent in these wing elements in both chick (Strong, 1902; Knopfli, 1919; Montagna, 1945; Fujioka, 1955) and quail at 7 d, although in the latter bird this ossification is minimal.

Table 3. *Stages in the development of the wing skeleton in the quail embryo as an aid to embryonic ageing*

Stage	Age of Embryo	Histology of the humerus	General histology of wing skeleton	Ratio of diaphysis to total bone length (humerus, radius and ulna) (approx.)	Ratio of humerus to wing length (approx.)	Rate of linear increase in wing skeleton segments
I	6 days	Immature cartilage	Cartilage in primary wing only	—	—	—
II	7 to 8 days	Mature cartilage, appearance of osteoblasts	Cartilage forming in manus region, osteogenesis in radius, ulna	1:3	1:4	High rate in humerus, ulna and manus segments
III	8 to 9 days	Calcification	Osteogenesis in metacarpals and phalanges	1:2	1:5	High rate in humerus, ulna and manus segments
IV	10 days	Cartilage, bone trabeculae	Osteogenesis in metacarpals and phalanges	2:3	1:4	Low increases in all segments
V	12 days	Osseous tissue prominent, cartilage degenerate	Osteogenesis in metacarpals and phalanges	2:3	1:4	High rate of increase in all segments
VI	13 to 16 days	Osseous tissue predominant, cartilage seldom seen	Most major elements with centre of ossification	2:3	1:4	General decline of growth rate in all segments

In the chick embryo of 5–10 d incubation 13 elements are identifiable in the carpal region (Montagna, 1945). Of these elements, four comprise a proximal row—the radiale, intermedium, ulnare and pisiform, for the centralia, and the remainder are distal carpals which never chondrify. In the quail embryo only six elements are seen as centres of early chondrogenesis in the 7 d embryo. Three are identified as radiale, intermedium and ulnare, and three as elements of the distal carpal series. Warren

(1934) reported that the carpal elements (unspecified) in the chick are cartilaginous at 7 d. There is general agreement that no ossification occurs in the carpal region of either chick or quail during the embryonic period.

The metacarpals of the chick appear in cartilage at 5–6 d (Montagna, 1945). Three elements are identifiable, Mc. II, III and IV. Metacarpals I and V are not seen at this stage but a vestige of Mc. V is recognisable in the poult as a rounded protuberance at the base of Mc. IV. Neither of these latter two elements is seen in the quail, but the remaining three appear in the order Mc. III and IV at 7 d, and II at 9 d. The order of ossification of the metacarpals in the chick is Mc. III at 6 d, IV at 7 d and II some time after hatching (Schinz & Zangerl, 1937; Montagna, 1945). In the quail, ossification commences in Mc. III and IV at 7–8 d with Mc. II still in cartilage at 16 d (pre-hatch stage).

Little close attention seems to have been paid to the development of the phalangeal region in the chick; however, Warren (1934) noted that these elements are cartilaginous at 8 d. This is approximately the same time as they are identified in the quail in pre-cartilage or cartilage. The appearance of centres of ossification in the phalangeal region is considerably earlier in the quail than has been reported for the chick. Schinz & Zangerl (1937) noticed that the initial centre of ossification appears at 12 d, whereas in the quail most major elements (including the phalanges) of the wing skeleton are ossifying by this time. The first centre of ossification to appear in the phalangeal region of the quail wing is at 9 d.

From a comparison of the development of the wing skeleton in the quail and the chick two points of interest emerge:

(1) The incubation period of the chick is 22 d as opposed to 16·5 d for the quail, but the wing skeleton of the chick starts to differentiate earlier than that of the quail.

(2) The elements of the primary wing skeleton of the chick commence development before those of the quail, but ossification is seen in the phalangeal region of the quail 2–3 d earlier than in the chick.

It seems likely that an acceleration occurs in the development of the quail wing at about 7–9 d with particular reference to the development in the metacarpal and phalangeal regions. Such an acceleration could result in an abbreviation in the development of the carpal region. This idea was first proposed by Montagna (1945) to explain the extensive fusions and deletions which occur in this part of the wing skeleton. He suggested that this region is subjected to compression from two rapidly differentiating and growing regions, i.e. the ulna/radius and the metacarpals. If this is the case, then it could be assumed that the deletions have gone a stage further in the quail with the elimination of the pisiform, centralia and the two distal carpal elements. Thus the basis for discrepancy between the number of elements identified by Montagna (1945) in the chick and those seen in the quail is explained.

SUMMARY

Differentiation of the wing skeleton in the quail commences with the appearance of centres of immature cartilage in the diaphyses of the humerus, radius and ulna at 5–6 d, and in the carpal, metacarpal and phalangeal regions at 7 d. Ossification begins at about 7–8 d and by 12 d most major bones in the wing are ossifying.

Histologically the humerus becomes osseous at 8–9 d incubation and this corresponds with a decline in its linear growth rate. A characteristic biphasic growth sequence is seen in the humerus, radius/ulna and carpal/metacarpal/phalanges. The peaks of maximum growth correspond to periods of maximum activity in the cartilaginous and osseous phases respectively.

The humerus, apart from a reduction at 8–9 d, maintains a near constant relationship in length to the whole wing and wing skeleton. The reduction in the linear growth rate at 8–9 d is correlated with a high growth rate in the metacarpal and phalangeal regions which have more recently commenced endochondral growth.

The sequence of growth is very similar to that in the chick except for a variation in the times of appearance of centres of chondrogenesis and osteogenesis in the various bones, suggesting an acceleration in the growth of the metacarpal and phalangeal regions which may account for a deletion of some of the carpal elements identifiable in the chick.

From the data presented a stage-by-stage sequence of development is constructed which is of use in the ageing of the quail embryo. This plan outlines criteria for six phases of growth relating specifically to the morphology of the wing skeleton.

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