A STUDY OF THE DEVELOPMENT OF THE "HAND" OF THE CHICKEN

by

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INTRODUCTION

Through the years, the development of the skeleton of the chicken has been the subject for considerable investigation. The method of the appearance and disappearance of certain bones in the distal portion of the wing, however, has not been clearly determined. This problem was undertaken for the purpose of restudying the development of the so-called "hand" of the chicken, Gallus domesticus (Ray).

ACKNOWLEDGEMENTS

Thanks are expressed to Dr. Mary T. Harman, Professor of Zoology, for suggesting this problem and for her helpful criticism, and to Dr. D. C. Warren of the Poultry Husbandry Department for furnishing the material on which the work was done.

REVIEW OF LITERATURE

Gegenbaur (1878) stated that the three digits of the chicken wing correspond to the first, second, and third of the pentadactyl hand, and that digits one and three had one phalanx each. Digit two had two phalanges. He claimed that in the early development of the wing there were four digits, but that the fourth disappears and that its metacarpal was found at the side of the third metacarpal. Parker (1888) agreed with Gegenbaur with reference to the metacarpals and digits. He also claimed that ossification began during the eighth day, and that in the thirty three-day old chicken, or twelve days after hatching, ossification began in the centro-ulnare and the carpal at the proximal end of metacarpal three. When the chicken was six weeks old ossification had begun in the intermedio-radiale and the carpal at the proximal end of metacarpal two. Parker stated that, "In a chicken three quarters of a year old the wing is still far from being perfect. The two large permanent carpal bones, those of the proximal row, alone remain distinct. The second and third carpals have united with one another and with their corresponding metacarpals. The carpal of digit one is now ossified. In the full grown

chicken, the outlines of all the distal carpals have become obliterated." Parker (1888) showed that in the six-day wing there were six carpals, and in the adult wing only two carpals remained as separate bodies. These carpals were the intermedio-radiale at the distal end of the radius, and the centro-ulnare at the distal end of the ulna. That the digits of the normal wing were the first, second and third, was claimed by Wiedersheim (1886). He stated that there were five carpal elements in the "hand" of the chick. Three of these elements were for the metacarpals, and the remaining two were found at the distal ends of the bones of the forearm. Wiedersheim (1886) stated, "Of the six or seven carpals which may be present in the embryo, the three distal ones become fused, and in the adult only the two of the proximal remain separate as a radiale and an ulnare." According to Bradley (1938), the digits were the first, second, and third, but he said that from embryological evidence the digits seemed to be the second, third, and fourth of the pentadactyl hand. From the conclusions of Kaupp (1918) and Sisson (1938), the digits were the first, second, and third.

The digits which remained were the second, third, and fourth of the pentadactyl hand according to Owen (1866). He also said that from geological evidence the ancient bird,

Archaeopteryx, had four digits which correspond to the first, second, third, and fourth of the pentadactyl hand. Lillie (1908) used Parker's drawing of the seven-day wing, but he called the digits the second, third, and fourth. He stated that in the six-day "hand", five digital rays grew out simultaneously from the carpal region. The first and fifth digits were smaller than the remaining three, and later disappeared leaving the second, third, and fourth digits. Lillie claimed that in the seven-day wing, three metacarpals remained representing the three permanent metacarpals, and digits two and four had two phalanges, and digit three had three phalanges. He also said that there were seven carpal bones. Four of these were in the proximal row, and three were in the distal row. Kingsley (1912) stated, "Development shows that the first digit is entirely lost and a fifth metacarpal, which is present in the early embryo, fuses early with the fourth, so that the digital formula is II, III, IV."

Concerning the carpometacarpus, Hyman (1922) stated, "The wrist bones are fused to the metacarpals to form the carpometacarpus, which consists of two elongated bones. The metacarpals contributing to these elements are the third and fourth. The second metacarpal is fused to the preaxial side of the proximal end of the third metacarpal

where it forms a pronounced hump. From this hump projects the second digit. The third digit is the longest and consists of two phalanges." The first and fifth digits were absent. Warren (1934) maintained that from embryological evidence the digits were the second, third, and fourth, and that the first and fifth digits were wanting. He also held that on the seventh day the metacarpals were represented by three cartilages which correspond to the three persisting digits of the wing.

Dawson (1926) described a method of staining the skeleton of cleared specimens with alizarin red S.. A technique to stain the cartilage of embryos with toluidine blue stain was developed by Bensley and Bensley (1938).

MATERIAL AND METHODS

The embryological material for this problem was furnished by Dr. D. C. Warren, Professor of Poultry Husbandry. About three hundred embryos were obtained, and of these two hundred and fifty wings were studied. Chick embryos ranging from six days to hatching age were used. The embryos were preserved in ninety-five percent alcohol.

Some of the wings were stained by the Dawson Method. This technique, although primarily a bone stain, shows cartilage fairly well, if the staining period is somewhat

shortened.

The material was first cleared by removing it from the alcohol, and placing it in a one percent solution of potassium hydroxide. When the connective tissue was rendered transparent, the specimen was removed from the clearing solution and placed in a fresh solution of the same clearing solution to which had been added enough of the alizarine red S. to make the solution a deep wine color. The specimen was allowed to remain in this solution until the ossified portions were stained a deep pink color. It was then removed, and placed in Mall's solution. This solution consists of one part glycerine to four parts of the clearing solution. The container was covered with tissue paper or cheese cloth so that the potassium hydroxide solution could evaporate. From time to time glycerine was added until a pure solution of glycerine was in the container. With this technique the ossified portions of the "hand" were well stained, but not the cartilaginous elements.

To stain the cartilaginous portions, the technique of Bensley and Bensley was used. The material was fixed in a solution of oxalic acid, formalin, and alcohol. It was then bleached in a one percent solution of ammonium hydroxide until it was snow white after which it was placed in the toluidine blue stain. The specimen remained in this

stain for three days until it was overstained, whereupon it was destained in acid alcohol. The destaining required seven days, and during this time the alcohol was changed daily. Destaining left the cartilage a deep blue and the connective tissue a light blue color. This technique resulted in the cartilage appearing in outline form, but the carpal elements were not distinguishable.

Another technique was devised whereby the material was cleared and stained at the same time by the Dawson Method. After the specimen was overstained it was destained in acid alcohol under a binocular-microscope. This technique gave the cartilage a brown color, and showed the carpal elements as separate bodies. Some very good results were obtained by allowing the material to remain in the stain and clearing solution until the connective tissue had separated from the cartilage. As this latter method left only the cartilage, it was easy to study the elements of the "hand". Study of the material was done entirely with the aid of compound and binocular-microscopes. The drawings were made by the use of a micro-projector.

OBSERVATIONS

The fore limb of the chick, in the six-day embryo, was a short outgrowth from the pectoral girdle. The "hand" was

at the distal end of this outgrowth and was spatulate in shape. All the elements of the arm, and of the "hand", were present in a cartilaginous condition. From the carpal region of the "hand" there extended five cartilaginous rays. These rays represented the metacarpals and the digits. The carpals could not be distinguished as separate bodies, but indentations in the cartilaginous mass indicated the point of division whereby the carpals would become separate bodies (Fig. 1).

To see the first digit in some of the specimens it was necessary to look on the palmar side of the "hand". Five digits were found in all the specimens. The development of the bones of the upper arm, and of the forearm, were not studied in detail, because of their similarity to those in other animals in which they are found. The rate of ossification in these bones was observed. No changes occur in them except that they grow in length and diameter as the embryo develops.

In the seven-day embryo the five digits were still present, and had grown larger (Fig. 2). It was observed that the carpals of the distal row were forming at the proximal end of their corresponding metacarpals, and the carpals of the proximal row were forming at the distal ends of the bones of the forearm.

Digits one and five were becoming smaller in the eight-day chick wing, and the three middle digits were becoming larger (Fig. 3). Ossification was also occurring in the bones of the forearm (Fig. 3). At this age there was a small cartilaginous projection at the distal end of the radius, and on the outer side of the second metacarpal (Fig. 3, I). A smaller projection was observed at the distal end of the ulna, and on the outer side of the fourth metacarpal (Fig. 3, V). These projections are the digits which are disappearing, namely the first and the fifth. There were seven cartilaginous elements present in the carpal region of the "hand" (Fig. 4). These elements were the three carpals at the proximal end of the three remaining metacarpals, the centrale, the ulnare, and the partly fused intermedio-radiale. The centrale and the ulnare were at the distal end of the ulna, and the intermedio-radiale was at the distal end of the radius. The carpal at the proximal end of metacarpal four was the result of the fusion which occurred between the carpal of the disappearing fifth metacarpal and the carpal of metacarpal four.

In some, but not all, of the specimens of this age ossification could be seen taking place in metacarpal three.

In the nine-day chick wing a change could be seen. Fusion of the centrale and the ulnare to form the centro-

ulnare reduced the number of carpal elements (Fig. 5, CU). The cartilaginous mass present at the proximal end of metacarpal four in the eight-day specimens could still be seen in the nine-day wing (Fig. 5, MC_5). In this stage ossification was pronounced in metacarpal three and four. Two carpals were larger than the others. The larger of these two, the intermedio-radiale, was at the distal end of the radius. The smaller, the centro-ulnare, was at the distal end of the ulna. The number of phalanges of the digits could be observed. Digits two and four had one phalanx each, and the third digit had two phalanges.

Little change was observed in the ten-day "hand" when compared with the nine-day (Figs. 5, 6). The third digit was becoming larger and longer as compared to the other two digits, and the metacarpals were ossifying. The number of carpals remained the same.

Fusion began to take place at the distal ends of metacarpals three and four on the 12th day. At this point of fusion there was a small ossified area from which extended the phalanges of digits three and four. Ossification was occurring in the phalanges of digit three, and the phalanx of digit two.

As the embryo grew older, the cartilaginous mass at the proximal end of metacarpal four gradually disappeared, and

by the time the embryo reached the 15th day it had disappeared.

In the fifteen-day "hand" there were five carpal elements in the region distal to the bones of the forearm, and proximal to the metacarpals (Fig. 7). These elements are the three carpals of the distal row, and the two carpals of the proximal row. Of the carpals in the distal row, the one at the base of metacarpal three was the largest, and of the proximal row, the intermedio-radiale was the larger.

Digit four was becoming ossified on the 18th day. However, this condition could not be seen in all the specimens of this age, but enough specimens showed this condition to determine that ossification was begining in that digit.

When the chick had reached the 20th day of incubation ossification was almost complete in the phalanges, the metacarpals, and the bones of the arm. No ossification had occurred in the carpals (Fig. 8).

Table 1. Average daily ossification from the 12th day to the 20th day of incubation.

Part ossified	Measurements of 130 wings	Amount of ossification
Humerus		0.92 mm.
Radius		0.75 mm.
Ulna		0.365 mm.
Metacarpal 3		0.655 mm.
Metacarpal 4		0.60 mm.

A study was made of the rate of ossification in the bones of the wings in 85 embryos. Measurements were taken from the 12th to and including the 20th day of incubation. As has been stated all the bones of the "hand", with the exception of the carpals and the phalanx of digit four, had started to ossify, and it was for this reason that measurements were started on the 12th day. The wings of 56 males and 29 females were studied for ossification. The results showed that the average rate of ossification per day was less than 1 mm. (Table 1).

Table 2. Average daily ossification by sex from 12th to 20th day of incubation.

Part ossified	Measurements of 72 wings (males)	Measurements of 58 wings (females)
Humerus	1.025 mm.	0.875 mm.
Radius	0.92 mm.	0.825 mm.
Ulna	1.26 mm.	0.425 mm.
Metacarpal 3	0.72 mm.	0.50 mm.
Metacarpal 4	0.245 mm.	0.42 mm.

When the rate of ossification by sex was made it showed that ossification progressed faster in the males than in the females (Table 2). In some bones the rate was almost twice as fast in the males as in the females. In the fourth metacarpal it was found that the females had a faster rate than did the males.

DISCUSSION

There was little agreement among investigators on the following four points: (1) the indentity of the three digits and their corresponding metacarpals; (2) the number of phalanges for each of the digits; (3) the number of carpal elements present; and (4) the time of ossification of the bones of the "hand".

Evidence in this study agreed with Lillie's (1908) findings that five digital rays were present in the six-day "hand". The projection described by Parker (1888) in the ten-day wing as "the accessory cartilage of the first metacarpal", was described by Lillie (1908) as being the rudimentary first digit. This was confirmed by the present findings, which also showed that there were five digits present in the seven-day wing. From the results of Lillie (1908) and Warren (1934) it seemed that by this time the first and fifth digits had disappeared and only three digits remained. Evidence in this study showed that the first and fifth digits were still present in the seven-day "hand", but that they disappeared later. Digits one and five disappeared by the eighth day, but the metacarpal of digit five persisted until the 13th day, and was fused with the metacarpal of the fourth by the 15th day. This metacarpal was

the rudimentary fourth as described by Gegenbaur (1878) and Parker (1888). This conclusion differed also from that of Kingsley (1912) who found that the fifth metacarpal fused earlier with the metacarpal of the fourth.

Results in this study agreed with those of Gegenbaur (1878), Parker (1888), and Hyman (1922) in that digits two and four had one phalanx each and digit three had two phalanges. These conclusions differed from the results of Lillie (1908) who claimed that each of the digits had one extra phalanx.

Parker (1888) differed from Lillie (1908) in that he showed six carpal elements in the seven day, and Lillie claimed that there were seven. Wiedersheim (1886) did not give a definite number of carpals, nor did he state the age at which the number he reported was found. The findings in this problem more nearly coincided with the results of Lillie (1908). In the eight-day "hand" there were seven carpal elements (Fig. 4), and in the fifteen-day there were five (Fig. 7). Some disagreement occurred upon the identity of the two carpals which remained separate. Wiedersheim (1886) called these carpals the radiale and the ulnare. The present findings agreed with those of the workers that considered these carpals to be the intermedio-radiale, and the centro-ulnare.

Concerning the initiation of ossification, the findings of Parker (1868) that ossification began in metacarpal three on the eighth day was confirmed in this study.

SUMMARY

1. In an investigation of the development of the so-called "hand" of the chicken, the wings of 125 chick embryos were studied.

2. In the six-day "hand" five digits were found. Two of these disappeared before the 10th day. They were the first and fifth. Thus the digits which remained were the second, third, and fourth.

3. Seven carpal elements remained in the eight-day "hand". Three of the seven were in the distal row, and four were in the proximal row. This number was reduced to five before the 15th day.

4. Ossification began in the third metacarpal on the eighth day. The rate of ossification was less than 1 mm. per day. Ossification in the males progressed at a faster rate than it did in the females. Metacarpals three and four were the first bones of the "hand" to ossify; the phalanges of digits two and three had begun to ossify before the phalanx of digit four.

5. The fifth digit was the first to disappear, and the

first digit was the last. The metacarpal of digit five persisted until the 15th day.

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EXPLANATION OF PLATE I

All the drawings, except Fig. 9, were made by the use of a micro-projector.

Fig. 1. Left wing of a six-day embryo. I, II, III, IV, V, digits; C, carpal region; R, radius; U, ulna; H, humerus. 20x.

Fig. 2. Left wing of a seven-day embryo. I, II, III, IV, V, digits; C, carpal region; R, radius; U, ulna. 15x.

Fig. 3. Left wing of an eight-day embryo. I, II, III, IV, V, digits; MC_3, third metacarpal; MC_4, fourth metacarpal; O, ossified portion; C, carpal region; P, phalanges; R, radius; U, ulna. 15x.

Fig. 4. Right wing of an eight-day embryo. II, III, IV, digits; MC_2, second metacarpal; MC_3, third metacarpal; MC_4, fourth metacarpal; MC_5, fifth metacarpal; DC, carpals of distal row; IR, intermedio-radiale; CE, centrale; UL, ulnare; P, phalanges; R, radius; U, ulna. 15x.

Fig. 5. Right wing of a nine-day embryo. II, III, IV, digits; MC_2, second metacarpal; MC_3, third metacarpal; MC_4, fourth metacarpal; MC_5, fifth metacarpal; DC, carpals of distal row; IR, intermedio-radiale; CU, centro-ulnare; P, phalanges; O, ossified portion; R, radius; U, ulna. 15x.

Fig. 6. Left wing of a ten-day embryo. II, III, IV, digits; MC_2, second metacarpal; MC_3, third metacarpal; MC_4, fourth metacarpal; P, phalanges; IR, intermedio-radiale; CU, centro-ulnare; DC, carpals of distal row; O, ossified portion; R, radius; U, ulna. 10x.

PLATE I

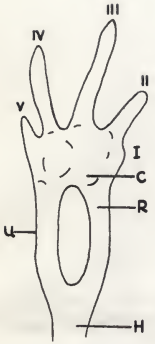


Fig. 1

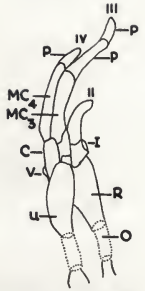
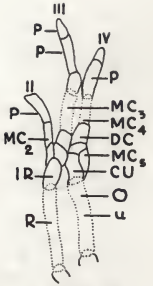


Fig. 3



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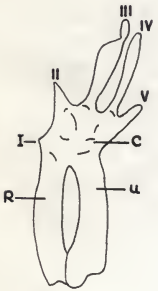

Fig. 2

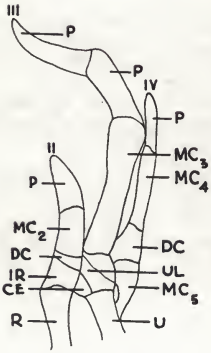

Fig. 4

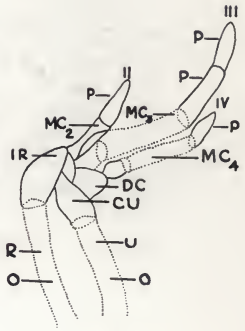

Fig. 6

EXPLANATION OF PLATE II

Fig. 7. Right wing of a fifteen-day embryo. II, second digit; MC_2, second metacarpal; MC_3, third metacarpal; MC_4, fourth metacarpal; IR, intermedio-radiale; CU, centro-ulnare; DC, carpals of distal row; R, radius; U, ulna; O, ossified portion. 26.6x.

Fig. 8. Left wing of a twenty-day embryo. MC_2, second metacarpal; MC_3, third metacarpal; MC_4, fourth metacarpal; IR, intermedio-radiale; CU, centro-ulnare; DC, carpals of distal row; O, ossified portion; R, radius; U, ulna. 18.6x.

Fig. 9. Adult wing. II, III, IV, digits; MC_3, third metacarpal; MC_4, fourth metacarpal; CM, carpometacarpus; IR, intermedio-radiale; CU, centro-ulnare; P, phalanges; R, radius; U, ulna. (From Bradley, 1938)