THE SKELETAL DEVELOPMENT OF THE ANTERIOR LIMB OF THE GUINEA-PIG, *CAVIA COBAYA* CUV., FROM THE 25-DAY EMBRYO TO THE 161-DAY POST NATAL GUINEA-PIG

by

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INTRODUCTION

Much work has been done on the development of the human skeleton. Some work has been done on birds and the albino rat, but very little research has been directed toward the bone development of the guinea-pig. This study represents a part of a series of studies on the embryology of the guinea-pig.

Purpose

The purpose of this research has been to determine the period in fetal life when ossification first begins, how it progresses, when it is completed, and how it varies in rate of development in opposite sexes, as well as to determine the retrogressive changes which occur in the formation of the foot.

Review of Literature

No literature was found on the normal bone development of the guinea-pig, but Sparks and Dawson (1928) in their study of the bone development of the albino rat noted that most of the centers of ossification are laid down in a relatively short time either in utero or in the period between birth and weaning.

Strong (1925) in working with the rat found that the clavicle is the first bone to ossify and that most of it is ossified at birth. He found, that in the ossification of the phalanges, that as in humans, the terminal phalanges are the first to ossify and the second phalanges the last. He also found that "although ossification takes place much more rapidly in the rat than in man, it never ceases while the animal is alive".

Pryor (1925) observed that in humans there are instances of normal early ossification in both sexes, and that there are normal cases in which ossification is delayed. In either case he believes that this is due to heredity. Apparently the order of ossification of the bones of the carpus is the same in both sexes. Pryor (1928) observed that the bones of a first child ossify, as a rule, sooner than those of subsequent children. In the female there is complete union of the epiphyses of metacarpal bones and the phalanges at sixteen years. In males in most cases this union is not completed until between 19 and 20 years of age.

Dawson (1927) describes the clearing and staining of skeletons with alizarin red. He developed this technique with rat material.

MATERIALS AND METHODS

The animals used in this research were secured through the courtesy and coöperation of Doctor H. L. Ibsen of the Animal Husbandry Department, Kansas State College. Most of the animals were from the colony described by Harman and Pritchett (1932), by Harman and Derbyshire (1932), Harman and Dobrovolny (1933), and Winters (1933). Besides this material, specimens were secured from Doctor Ibsen. These

were the animals used in his paper on the prenatal growth in guinea-pigs (Ibsen, 1928).

A total of 70 animals was examined. Of these, 21 were males, 25 were females, and the sex of the remaining 24 was not identified. This included animals between the ages of 25 day embryos, copulation age, and 161 days post natal development. The age of the embryos was determined by the method described by Harman and Prickett (1932) and Winters (1933). The sex in all specimens was determined by external genitalia.

Gross anatomical dissections were studied together with transparent specimens which were prepared by the process described by Dawson (1926). By use of this process the flesh and cartilage were rendered transparent and the bones were stained with alizarin red. When these cleared specimens were placed in a Petri dish over a photographic printing box, the light penetrated the tissue so that the very slightest ossification could be detected. The younger animals were examined with a binocular microscope. The bones of animals between the ages of 40 day embryos, copulation age, and 75 days post natal development taken at intervals of five days were dissected from the other tissues and measured with calipers to determine the amount of ossification at the various stages.

Some of the material was fixed in 7 per cent formalin, some in Bouin's fluid, and some in 85 per cent alcohol. Illuminating gas was found the most satisfactory for killing the animals of post natal development and the pregnant mothers.

The fetal membranes were removed from the embryos. Then the smaller embryos were fixed, cleared and stained in toto. In the larger embryos and in the post natal animals one front appendage was removed from the body. The skin, most of the flesh, and the fat were cut away. This was done so that the clearing process would be more simple. The other appendage was saved for reference.

OBSERVATIONS

Since no record of the anatomy of the skeleton of the guinea-pig was found, it was necessary to make a study of the anatomy of the bones of the front leg.

Anatomy of the Forelimb

The forelimb of the guinea-pig consists of the scapula, clavicle, humerus, radius, ulna, eight carpals, four digits, each of which consists of a metacarpal and three phalanges; and a rudiment of a fifth digit which is composed of two bones (Fig. 4). It is assumed, in this case, that the two

bones are a rudimentary metacarpal and one phalanx (Fig. 4, FM and PH). A number of sesamoid bones are found on the volar surface of the foot (Fig. 2, S and S^1). We find that in each of the four digits there are two sesamoid bones which lie parallel to one another at the point of the articulation of the metacarpal and the first phalanx (Fig. 2, S). These two bones are parallel to the phalanges. In addition to these there is another sesamoid bone on each of the four digits, which extends crosswise where the distal phalanx articulates with the second phalanx (Fig. 2, S^1). A small sesamoid bone which corresponds to the pisiform lies ventral to the proximal end of the fifth metacarpal. In the adult there is a sesamoid bone in the walking pad of the foot just ventral to the fourth and fifth metacarpals.

The carpals are arranged in two rows, a proximal row of three bones and a distal row of five. In the proximal row from the medial (thumb or radiale side) to the lateral side, the following bones are found: radiale, lunare, and ulnare; and in the distal row following the same order the trapezium, trapezoid, centrale, capitate, and hamate are present (Fig. 4). The clavicle is very small. It is a slender curved rod of bone imbedded in the muscle of the shoulder. The scapula is a flat bone with a spine on the dorsal surface. The spine unites with the body of the scapula from the prox-

imal edge of the body for a distance which is approximately half the length of the scapula.

How Ossification Takes Place

The first bone to show ossification is the clavicle. A slight ossification about the size of a pin point was observed in the embryo of 25 days copulation age. In the next three days there was very little change in ossification. No other centers of ossification appeared, and there is only a slight increase in the ossification of the entire bone. In embryos from 28 to 31 days copulation age, centers of ossification appear in the scapula, radius, ulna and humerus. Ossification in the radius, ulna and humerus appears as a center located at the middle of each of the diaphyses. In each case the ossification center increases rapidly in density and extends toward both ends. In embryos of 31 days copulation age ossification has progressed to the stage where about half of the cartilaginous fundament of the shafts is completed in the long bones. The scapula has a large ossification center which includes most of the body and the spine. The spine extends well above the body of the scapula, but the acromion and coracoid processes cannot be distinguished at this early stage.

In the 35 day embryo ossification centers were observed in the second, third, and fourth metacarpals, as well as in the distal phalanges of the second, third, fourth and fifth digits. The coracoid process has started to form.

In embryos of 40 days copulation age a center of ossification has appeared in the shaft of the fifth metacarpal (Fig. 1, M^1), and one in the first phalanx of each of the second, third, fourth and fifth digits (Fig. 1, P). The areas undergoing ossification in the radius, ulna, and humerus have elongated and take a heavier stain at each end. The scapula takes a deep stain around the region of the glenoid cavity and in the region just above it in the acromion process. This indicates that ossification is taking place. The clavicle has not progressed beyond the stage observed in the 35 day embryo. The pollex is folded over the volar surface of the foot, but the distal end is still free and projects out beyond the surface of the foot.

In the 45 day embryo, centers of ossification have appeared in the second phalanx of the second, third, fourth and fifth digits. The areas ossified in the metacarpals have elongated considerably.

In the 51 day embryo two small centers about the size of a pin point are lying side by side in the distal epiphysis of the radius. The distal and proximal epiphysis of the

ulna each have two centers of ossification. The upper epiphysis of the humerus has in the lateral side two small centers of ossification which lie side by side, and a larger ossification center in the lower epiphysis. A center of ossification appears in the radiale, and one in the humate. There is a center of ossification on the distal margin of the acromion process. This large center of ossification is uniting with the body.

In the 55 day embryo there is a secondary center of ossification present in the radiale (Fig. 3, RA), and the ulnare (Fig. 3, U), and a primary center of ossification in the lunare (Fig. 3, L). In the first phalanx in each of the second, third, fourth, and fifth digits a center of ossification is found in the proximal epiphysis. A center of ossification is found in the capitate (Fig. 3, C), and one in the proximal epiphysis of the radius (Fig. 3, EP).

In the 60 day embryo the centrale, the trapezoid, the trapezium and the first metacarpal each has an ossification center. The two small centers of ossification which were observed in the proximal epiphysis of the humerus in the 51 day embryo have united and form the greater tuberosity of the epiphysis in the proximal extremity of the humerus (Fig. 3, GE). Another center has appeared which forms the lesser tuberosity. There was observed a center of ossifica-

tion or plate distal to the radiale. Of the two sesamoid bones which lie on the volar surface of the foot in the adult at the articulation of the metacarpal and the first phalanx (Fig. 2, S) the one on the lateral side of each digit has a center of ossification which includes about half of the cartilaginous fundament. Both ossification centers in the ulnare have enlarged but they remain apart. The clavicle is ossified except at the extremities where growth is occurring. There is a center of ossification in the phalanx of the pollex or the first digit.

In the 65 day embryo there was observed a center of ossification in each of the second or medial sesamoid bones which lie on the volar surface of the metacarpal-phalanx articulation, and a center of ossification in each of the sesamoid bones on the articulation of the second and third phalanges (Fig. 2, S^1). There is an ossification center in the cartilage on the margin of the acromion process.

Ossification After Birth

At birth the epiphysis of the proximal extremity of the humerus is still divided. The two centers of ossification in the ulnare are separated by a comparatively wide margin. All of the epiphyses of the phalanges are free from the shafts. The clavicle is about 3.3 mm. in length. Two

centers of ossification in the distal extremity of the third phalanx are located on each side, lateral and medial. These give the claw an appearance of the toes on a cloven foot of some of the higher vertebrates (Fig. 4, FI).

In the guinea-pig of 13 days post natal development, another center of ossification was observed in the cartilage bordering the acromion process. The centers which had been observed previously had united with the body of the process. The humerus has a center of ossification in the medial epicondyle. The head of the radius which is quite flat is free from the shaft.

In the guinea-pig of 17 days post natal life, a center of ossification was observed in the cartilaginous fundament bordering the acromion process. In the guinea-pig of 18 days post natal life an ossification center was observed in the same position, and between this ossification center and the body of the process were found three very small centers in a row parallel to the large center and the body. In this animal, for the first time, we observed an ossification center in a cartilage ventral to the proximal end of the fifth metacarpal. This apparently is the pisiform. This bone was found in animals of 26 days, 75 days, 95 days of post natal life and in the adult, but in all cases it remained very small (about 1 mm. in length).

In the guinea-pig of 75 days post natal life, we observed a center of ossification in the cartilage bordering the acromion process. The point on the process is still very blunt. There was observed a center of ossification in each the dorsal and ventral sides of the cartilaginous fundament on the vertebral margin of the scapula. The two centers in the ulna have united.

In the guinea-pig 161 days after birth, we observed a center of ossification in the cartilaginous fundament on the lateral side of the walking pad formed by the folding over of the first digit. This is apparently a sesamoid bone formed in the tendon which is found fastening the walking pad to the lateral side of the foot. All of the epiphyses were free from the diaphyses. It was noted that the centers of ossification in the cartilaginous fundament on the vertebral edge of the scapula extended almost the entire distance from the ventral to dorsal sides. The dorsal projection of the acromion process was quite short compared with the projection found in the adult guinea-pig.

Ossification was not complete in any of the bones except the clavicle.

In the adults which were examined in each case we found that the epiphyses had not made complete union with the diaphyses. The most apparent example of incomplete ossifica-

tion in every case could be observed in the ossification and union with the body of the vertebral margin of the scapula. In the oldest animal examined which had reached the age of two years, 11 months and 10 days, there was a distinct line between the vertebral margin and the body of the scapula.

Variation in Rate of Ossification of Bones in the Opposite Sexes

Beginning with embryos of 31 days copulation age and taking two of each age at five day intervals until birth (68 days) and of animals after birth at about five day intervals until 45 days post natal life, we observed the development with special reference to sexual differences in rate of ossification. In case of the smaller embryos both males and females of the same age were from the same litter thus providing the same nutriment and growth conditions. In most of the older animals the males and females of the same age were from different litters.

In no case did we observe any significant differences in the rate of development of ossification in the opposite sexes. The centers of ossification were noted to appear in both males and females at the same ages, and no difference in the degree of ossification was noted.

. In general there was noted a gradual increase in the length and width of the bones of the animals which were examined.

There were exceptions in cases of the animals of 26 days and 35 days of post natal life. The male of 26 days post natal life was larger than the female of the same age, and ossification of the bones in the male was in advance of those in the female. In the case of the animals of 35 days of post natal life the reverse condition was true. In this case the female was larger and ossification of the bones was in advance of that in the male. Since these animals though of the same age were from different litters, it appears that these variations are probably due to individual differences.

Retrogressive Changes

Five digits in the appendages are characteristic of mammals. In the 25 day embryo of the guinea-pig four digits are present as clearly outlined projections and the fifth digit appears on the ventral surface of the foot in the thenor eminence as a bump about the size of a pin head. In the adult only four digits are recognized externally. The remains of the fifth are found on the volar surface of the foot imbedded in the posterior walking pad. When it is

dissected from the rest of the tissue it lies back in the position which corresponds to the thumb in the hand. From the position which it occupies it is assumed that this rudimentary digit is the pollex or the first digit. It articulates with the radiale. It is composed of two bones, a rudimentary metacarpal and one phalanx. The metacarpal has been modified into a small flat ovoid bone. The phalanx is about as long as the second phalanx in the other digits, but it is flattened and the distal end remains cartilaginous. No sesamoid bones are present in this digit.

The ulnare, although considered a part of the proximal row of carpals, occupies a position ventral to the rest. The clavicle which is quite large in some of the mammals is very small in the guinea-pig and apparently does not perform a definite function. In the adult it is only 10 mm. long.

DISCUSSION

The foregoing data show that as in the rat as described by Sparks and Dawson (1928) most of the primary centers of ossification are laid down early. The clavicle which is very small is the first to ossify which fact Sparks and Dawson (1929) also found true in the rat. The first phalanges are the first to show ossification centers

and the second phalanges are the last. This differs from the rat since Sparks and Dawson (1928) report that the terminal phalanges were the first to ossify.

The period between 28 and 31 days gestation in the guinea-pig in which many of the primary centers of ossification are laid down coincides with the period of rapid development of external form as described by Harman and Dobrovolny (1933) in which they state, "After the 29th day the length of the fetus and its component parts increases rapidly ... The length of the fetus at 31 days is 25.9 mm., a marked increase over the length at 29 days".

The ossification of the metacarpal and the phalanx in the first digit takes place relatively late. This is probably due to its rudimentary nature.

With a few exceptions at least two animals of each age were examined. Of these one was a male and one a female. In many cases three or four specimens of the same age were examined. In the case of the animals of 75, 95 and 161 days of post natal life only one animal of each age was examined. In the embryos of 25, 26, 27, 28, 29 days of gestation no positive claim of recognition of the sex of the embryo could be made. Although several embryos of each of these ages were examined no comparison of sex differences were made.

Some of the specimens which were discards from other experiments and had been fixed in Bouin's fluid were used for dissection only. Material which had been fixed in Bouin's fluid could not be cleared until all of the fixative had been washed out. This necessitated a prolonged process with a greater chance of maceration of delicate tissue. The presence of picric acid in Bouin's fluid causes a degree of decalcification and destruction of some of the smaller centers of ossification which would lead to inaccurate results. For this reason all the younger embryos which were cleared and stained were fixed in 85 per cent alcohol for 24 to 48 hours and in 95 per cent alcohol for 96 hours before clearing in a 1 per cent solution of potassium hydroxide.

SUMMARY

1. The age and order of appearance of the centers of ossification for each bone of the front leg of the guinea-pig have been determined.

2. The clavicle is the first bone to show ossification which at 25 days gestation has a center of ossification about the size of a pin point. The clavicle is completely ossified at birth except at the extremities where growth occurs.

3. The next bones to show ossification centers are the scapula, the humerus, the radius, and the ulna. These appear in the period between 28 and 31 days gestation.

4. In the embryo of 35 days copulation age centers of ossification appear in the metacarpals, and in the terminal phalanges.

5. The coracoid and acromion processes ossify by means of several ossification centers which are formed successively. They form one at a time in the cartilaginous fundament on the margin of the ossified area and spread until they unite with the body.

6. All the primary centers of ossification of all the bones with the exception of a few sesamoid bones are present in the embryo of 60 days copulation age.

7. Ossification was not complete in any of the animals examined.

8. No differences in the rate of ossification of bones of males and females were noted in embryos between the ages of 31 days gestation and birth (68 days) or animals of one day and 45 days of post natal life.

9. Retrogression has occurred in the formation of the foot. There was a beginning of five digits on the foot of the embryo and only four digits are present in the adult.

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The author wishes to express her indebtedness to Dr. Mary T. Harman for the assistance, suggestions and criticisms given her while carrying on this research, and to Dr. H. L. Ibsen of the Animal Husbandry Department for his coöperation in furnishing the animals for the experiment; also, to Marjorie Prickett Dobrovolny for her assistance in the identification of the sex of the embryos, and to other members of the Zoology Department for their assistance in methods of technique.

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EXPLANATION OF PLATES

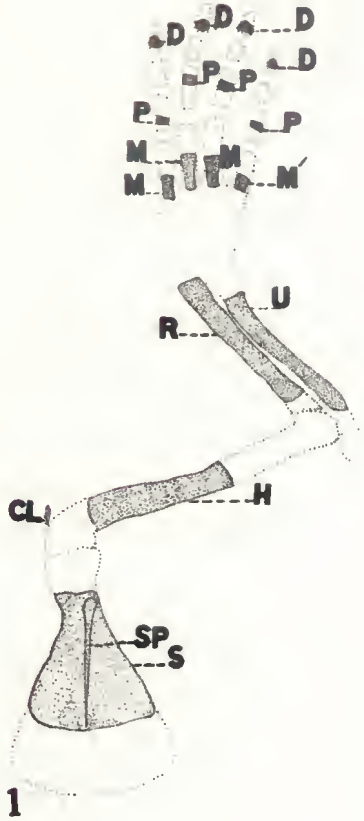
PLATE I.

Fig. 1. Right forelimb of the 40 day embryo. X3. The stipled area represents the extent of ossification. D, distal or third phalanges; P, first phalanges; M, the metacarpals; M^1, the fifth metacarpal; R, radius; U, ulna; H, humerus; CL, clavicle; S, scapula; SP, spine of scapula.

Fig. 2. Volar surface of the left foot of a female 75 days old. X2½. S and S^1, sesamoid bones; P, posterior walking pad; C, cartilage of first digit; M, metacarpal of first digit; FP, phalanx of first digit; RA, radiale; UL, ulnare; R, radius; U, ulna.

Fig. 3. Left fore limb of a 55 day embryo (male). X3. The stipled area indicates the portion that is ossified. C, capitate; H, hamate; RA, radiale; L, lunare; U, ulnare; EP, distal epiphysis of the radius; EU, distal epiphysis of ulna; CAP, capitulum; PE, proximal epiphasis of ulna; GE, greater tuberosity of humerus; AP, acromion process.

PLATE I.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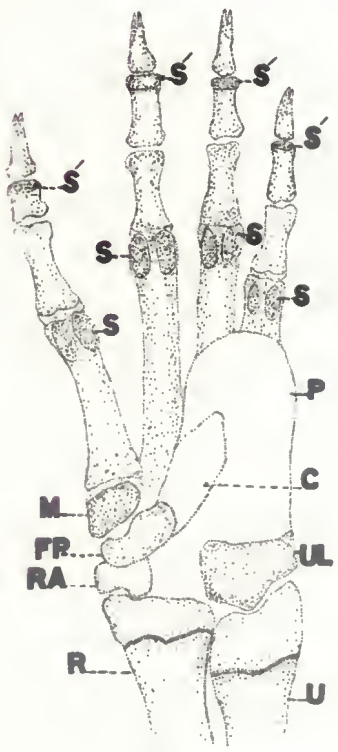
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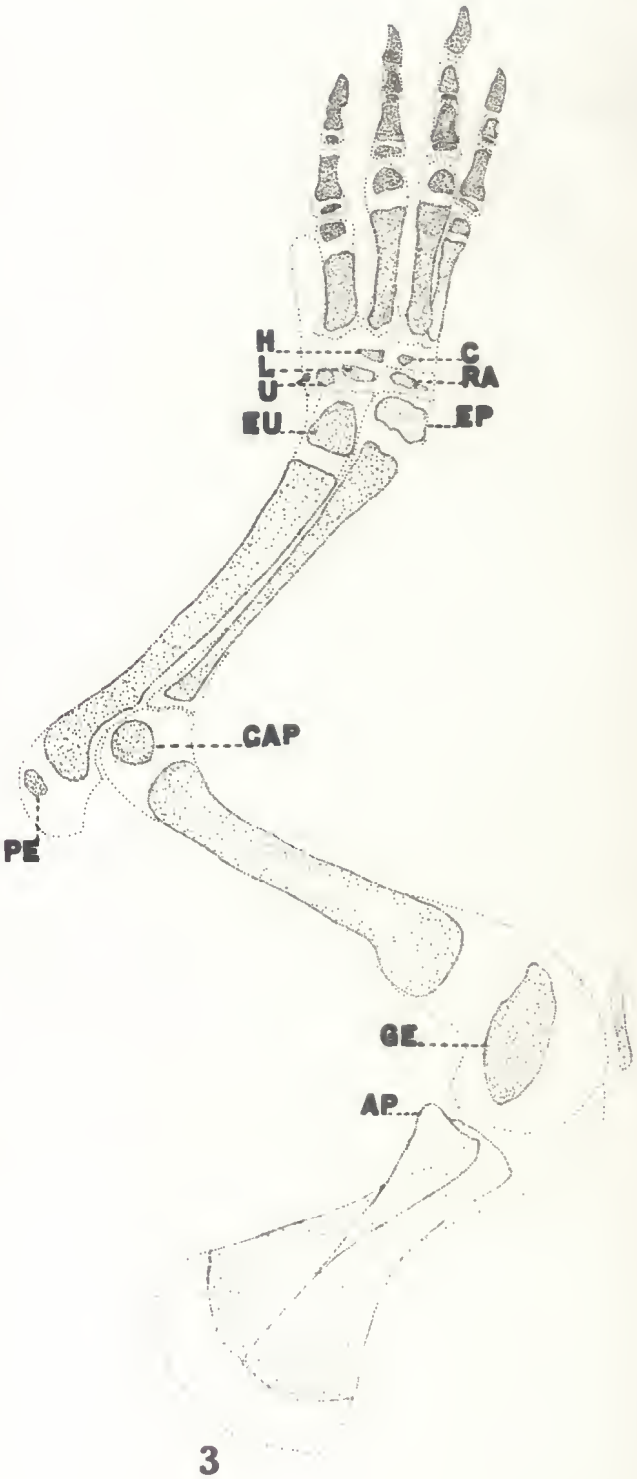
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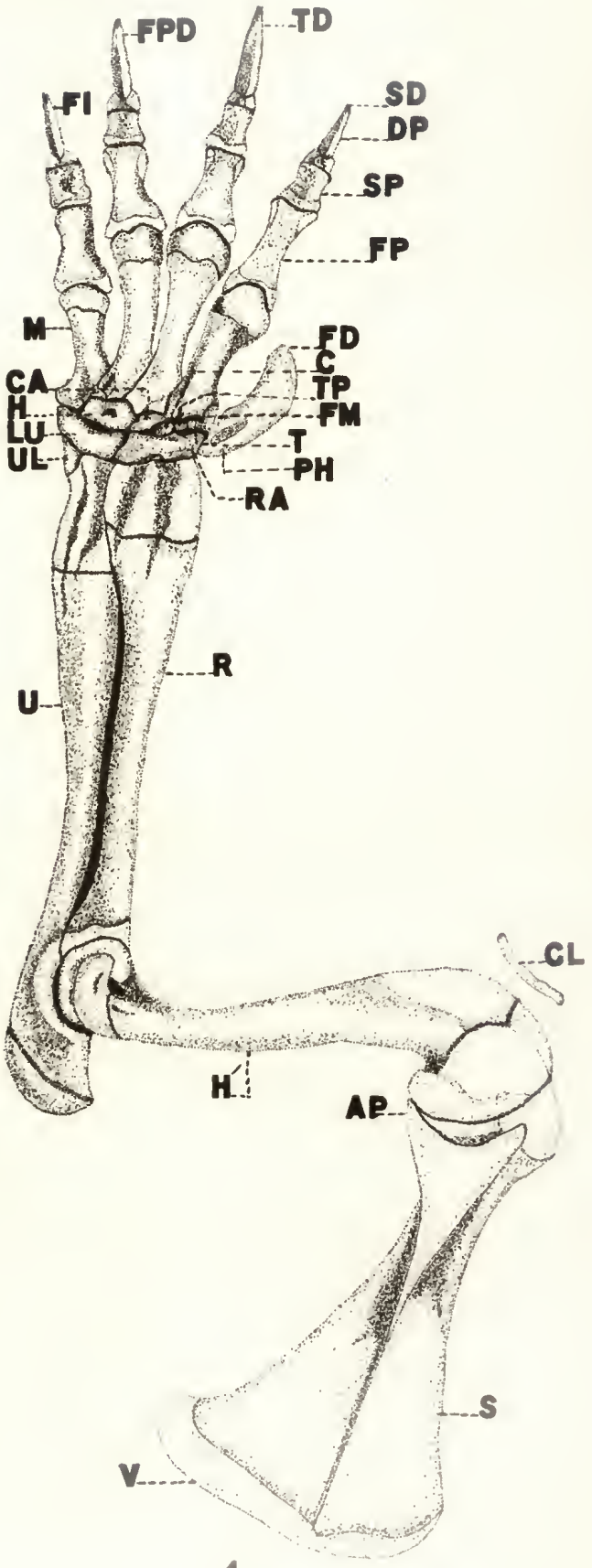
PLATE II.

Fig. 4. Left front leg of a guinea-pig one day after birth. X3. The first digit which normally would not be seen in the dorsal view has been folded back so that is is shown here. SD, second digit; DP, distal phalanx; SP, second digit; FP, first phalanx; FD, first digit; C, centrale; TP, trapezoid; FM, first metacarpal; T, trapezium; PH, phalanx of first digit; RA, radiale; UL, ulnare; LU, lunare; H, hamate; CA, capitate; M, metacarpal; FI, fifth digit; FPD, fourth digit; TD, third digit; R, radius; U, ulna; CL, clavicle; H^1, humerus; AP, acromion process; S, scapula; V, vertebral cartilage of scapula.

PLATE II.



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