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ABSTRACT

Two hundred forty-two specimens of *Onychomys leucogaster*, ranging in age from day of birth to the twenty-eighth day were cleared and stained using both Alizarin KOH and Alcian Blue/Alizarin-Trypsin staining methods. Thirty centers of ossification were studied. The data demonstrate the following: 1) skeletal centers of the appendicular skeleton ossify and mature earliest; 2) a new sesamoid bone lateral to the distal condyles of the femur was discovered; 3) the skeletal ossification of the baculum is present at one day of age; 4) a high degree of individual variation precludes aging of this species by skeletal maturation.

INTRODUCTION

In mammals, much of the completion of skeletal ossification occurs postnatally (Romer and Parsons, 1978); however, few studies have examined this process in detail (Nesslinger, 1956; Kirkland, 1970, 1973). Techniques have been available to clear and then stain the skeletal elements of a neonatal specimen with Alizarin Red (Nesslinger, 1956; Humason, 1979). Recently, use of Alcian Blue to counterstain cartilage has been demonstrated (Dingerkus and Uhler, 1977). Using these techniques, detailed analysis of skeletal ossification can be conducted.

This study was undertaken to determine the patterns and rates of postnatal ossification in the northern grasshopper mouse, *Onychomys leucogaster*. From these data, a better understanding of skeletal development in association with the appearance of specific locomotor activities can be elucidated. In addition, an attempt to use postnatal skeletal development as an early aging technique in this species was examined.

METHODS AND MATERIALS

Specimens used in this study were obtained from a colony of northern grasshopper mice maintained in the Basic Animal Services Unit of the University of Arkansas at Little Rock. Original members of the colony were captured in Greer and Harmon counties, Oklahoma. Mice were maintained in the laboratory on Purina Formulab #5008 and provided water *ad libitum*. Preserved specimens from the study are deposited in the UALR Vertebrate Collections.

Groups of mice (consisting of 11 individuals) were taken from day of birth to day 14 and then every other day until day 28. Of these 242 specimens, 220 were sacrificed, cleared and stained by the Alizarin-KOH method (Nesslinger, 1956; Humason, 1979). In order to examine ossification in conjunction with existing cartilage, the remaining 22 specimens were sacrificed, cleared and stained by the Alcian/Alizarin-Trypsin method (Dingerkus and Uhler, 1977). All specimens were subsequently stored in glycerol.

The specimens were examined using a standard American Optical binocular dissecting microscope with a zoom lens. Observations were done qualitatively, noting the presence, absence and degree of ossification.

RESULTS AND DISCUSSION

A total of 30 centers of ossification were examined in detail. These particular centers represented components of both the axial and appendicular skeletons and were chosen for study because they represented critical areas with the least amount of ossification at birth. The results of the study are summarized in Table 1 and Figures 1-5. Based upon the results presented in these figures and table, the 30 centers of ossification are examined in the following discussion.

Axial Skeleton:

Occipital Region

The occipital region forms the posterior surface of the skull. It fuses anteriorly with the parietal bones and laterally with the temporal region. Growth and ossification of this region was noted for dorsal fusion with the interparietals and laterally with the temporal region. Fusion with the interparietal occurred first. This fusion consisted of finger-like projections that bridged the gap between the two bones and was completed by day 8. Lateral fusion took longer as the occipital region grew to meet the temporal region. Fusion in this area was completed by day 12. Fusion in both areas appeared to be caused by the growth of the occipital as neither the interparietal nor the temporal region seemed to extend toward the occipital.

Vertebral Column

The vertebral column plays an important role in mammals, being essential for protection of the spinal cord and nerves and giving support to the body. At birth, the vertebrae are composed of one ventral and two lateral plates. The lateral plates grow dorsally and fuse to form the neural spine. The ventral plate grows dorso-laterally to meet and fuse with the two lateral plates. The vertebral column is divided into five regions: cervical, thoracic, lumbar, sacral and caudal. Each of these five regions was studied independently.

Cervical — As in most animals, there are seven cervical vertebrae. At birth, the vertebrae are complete cartilagenous rings where calcium is eventually deposited. The order of fusion and ossification in this region is: vertebra 4, 7, 3, 5, 6, 2, 1. Complete ossification of some vertebrae is first seen on day 10, but the region is not completed until day 18.

Thoracic — Ossification of the 13 thoracic centra is completed by day 14, however, only one of the neural spines (on the second vertebra), characteristic of this region, was formed by day 28. The order of final ossification in this region is: 5, 4, 3, 6, 7, 8, 9, 10, 2, 1, 11, 12, 13.

Lumbar — The six lumbar vertebrae show signs of ossification on day 8 and are completely ossified by day 18. These vertebrae ossify in descending order.

Sacral — There are four sacral vertebrae in the grasshopper mouse. While eventually fusing completely to form the dorsal surface of the pelvic girdle, fusion had only occurred in the first two sacral vertebrae by day 28. Fusion of sacral vertebrae is also in descending order.

Caudal — The number of caudal vertebrae ranges from 6-15 averaging 14. Only the first three have neural crests and these were ossified by day 8 in descending order.

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Table 1. Postnatal ossification of the northern grasshopper mouse, *Onychomys leucogaster*. Numbers indicate the number of specimens in each age group which demonstrate ossification.

Center of Ossification	Age in Days																					
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	16	18	20	22	24	26	28
Axial Skeleton																						
Occipital Region	-	-	-	-	3	6	8	9	10	10	10											
Cervical Vertebrae	-	-	-	-	-	-	-	-	2	5	3	5	8	8	9	9	10	10	10			
Thoracic Vertebrae	-	-	-	-	-	-	1	-	6	9	4	9	8	8	10	10	10					
Lumbar Vertebrae	-	-	-	-	-	-	-	-	5	7	5	7	8	8	10	10	10					
Sacral Vertebrae	-	-	-	-	4	7	10	10	10													
Caudal Vertebrae	-	-	-	-	1	8	10	10	10													
Sternum	-	-	-	-	-	-	-	-	2	1	-	9	10	10	10							
Appendicular Skeleton																						
Carpal #1	-	-	-	-	2	2	6	5	8	10	10	10										
Carpal #2	-	-	-	2	2	2	6	3	8	10	10	10										
Carpal #3	-	1	-	-	2	3	6	6	8	10	10	10										
Carpal #4-5	-	1	-	8	7	10	10	10														
Centrale	-	-	-	7	6	10	10	10														
Radius-Intermedium	-	-	-	3	2	7	6	7	9	10	10	10										
Ulnare	-	-	-	5	2	10	10	8	9	10	10	10										
Sesamoid	-	-	-	-	2	1	7	3	9	10	10	10										
Pisiform	-	-	-	1	2	5	8	7	9	10	10	10										
Olecranon	-	4	-	8	10	10	10															
Tarsal #1	-	-	-	6	4	6	-	-	7	10	10	10										
Tarsal #2	-	-	-	-	-	-	-	-	8	10	10	10										
Tarsal #3	-	-	-	-	1	1	-	3	8	10	10	10										
Tarsal #4-5	-	-	-	2	3	8	9	9	9	10	10	10										
Astragalus	-	6	8	10	10	10																
Calcaneus	7	10	9	10	10	10																
Tibiale	-	-	-	-	-	-	-	-	9	10	10	10										
Centrale	-	1	-	5	4	2	4	6	9	10	10	10										
Tibia-Fibula	-	-	-	3	5	10	10	10														
Patella	-	-	-	-	-	-	-	-	1	3	-	-	4	3	1	4	10	10	10			
Distal Femur	-	-	-	-	-	-	2	1	7	10	9	9	10	10	10							
Pelvic Girdle	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	10	10	10	10
New Sesamoid Bone	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	10	10	10		

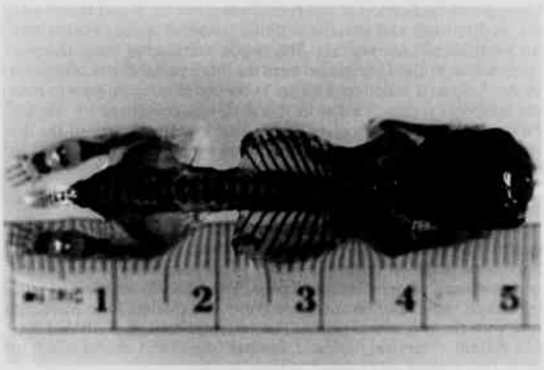

Figure 1. Dorsal view of 4 day old grasshopper mouse. Note degree of ossification of the vertebral column and pelvic region.

Figure 2. Lateral view of 9 day old grasshoper mouse. This age group represents the greatest single day completion of ossification centers (see Table 1).

Sternum

The sternum of the grasshopper mouse, like many other mammals, has six elements, all of which are present at birth. The elements, however, differ in size with the fifth sternal element being smallest. In several specimens, the fifth element at birth consisted of two or three small specks. Nesslinger (1956) found multiple specks replacing the fourth and fifth sternal elements in his work with the Virginia opossum. He reasoned that this area has multiple sites of ossification which subsequently fuse together to form the fifth sternal element as seen in the adult.

Appendicular Skeleton:

Wrist

The wrist of the grasshopper mouse is composed of nine bones: carpals 1-5 (four and five being fused), centrale, radius-intermedium, ulnare, pisiform, sesamoid. Complete ossification in this area is accomplished by day 10. The order of ossification is: centrale, carpal 4-5, ulnare, pisiform, radius-intermedium, sesamoid, carpal 3, carpal 1, carpal 2.

Olecranon Process

The olecranon is proximal to the semilunar notch of the ulna. While absent at birth, the olecranon is seen as early as day 1 and is fully ossified in all specimens by day 4. This process was expected to ossify early because of its important for muscle attachment.

Ankle

The ankle is composed of eight bones: tarsals 1-5 (four and five

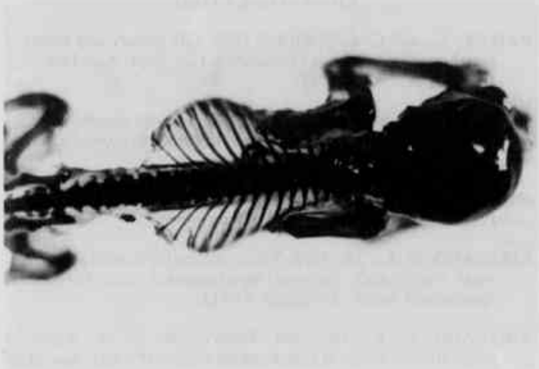

Figure 3. Dorsal view of 14 day old grasshopper mouse. Note ossification of vertebral column and pelvic region. By this age, 77% of the ossification centers studied were completed.

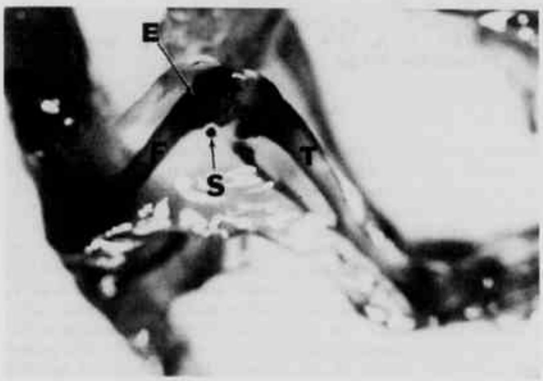

Figure 4. Hind limb of 28 day old grasshopper mouse showing new sesamoid bone (S). The femur (F) and tibia (T) are labelled for reference. Note the distinct epiphyseal plate (E) of the distal femur.

being fused), astragalus, calcaneus, tibiale, and centrale. The calcaneus is the first bone to appear, being present at birth. All components of this area are ossified in the following order by day 10: calcaneus, astragalus, tarsals 4-5, centrale, tarsals 1-2-3, and the tibiale.

Tibia-Fibula

The tibia and fibula are closely associated bones in mammals. In most rodents, this close association leads to fusion along all or part of their lengths (Romer and Parsons, 1978). This partial fusion is also present in the grasshopper mouse. The fusion of the two bones begins on day 5 as net-like strands across the notch at the proximal crossing of the bones. The fusion proceeds distally until the distal end of the fibula is met, after which the two bones cannot be distinguished along the area of fusion. The proximal ossification of the tibia and fibula differ greatly with respect to their time of appearance. Ossification of the tibia appears on day 9, while ossification does not appear until day 14 on the fibula.

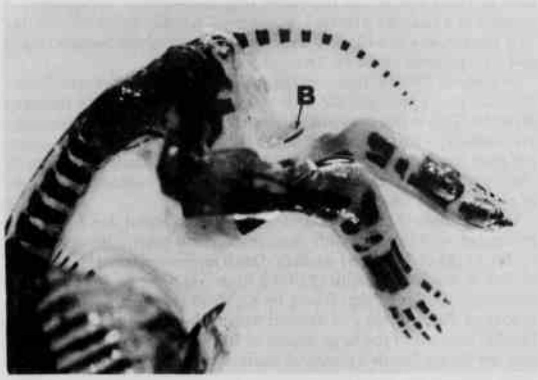

Figure 5. Posterior region of 6 day old grasshopper mouse showing the baculum (B).

Patella

The patella is a sesamoid bone that aids in the protection of the knee joint. At birth, the patella is observed as a sheet of cartilage covering the knee. Ossification is first seen as a small speck of bone on day 18 and is complete by day 26. As the patella is not as essential as other bones in the leg, the delay in its ossification is not unexpected.

Femur

The shaft of the femur in grasshopper mice, as in other mammals, is separated from the condyles by the epiphyseal cartilage. The separation of the distal condyles from the shaft makes it an ideal point for study, as growth and ossification can easily and accurately be observed. The condyles are formed in several ways (Kirkland, 1970). The mode of formation in the grasshopper mouse is via a bi-lobed or dumbbell shaped growth, first appearing as a tiny speck about day 8. The condyles subsequently grow proximally until they meet the epiphyseal cartilage at day 12.

Pelvic Girdle

At birth, the three bones of the pelvic girdle, ilium, ischium and pubis, are separate and distinct rod-like bones located on either side of the animal. Two points of fusion can be observed in this region. First, and most important, is the fusion of the three bones forming the acetabulum. The second point of fusion is that of the ischium and pubis, forming the obturator foramen. Completion of the pelvic girdle does not occur until day 22. However, Alcian stained specimens show that the region is complete at birth and that calcium is deposited later. The fact that the structure is complete, although not yet calcified, may be due to the importance of the pelvic girdle in support and locomotion. Fusion of the pelvic girdle and sacral vertebrae was not complete at day 28.

New Sesamoid Bone

A previously undescribed sesamoid bone, lateral to the distal condyles of the femur, was discovered in this study. The bone (Fig. 4) was first seen on day 20 and was thought to be a staining artifact. However, closer observation revealed a small depression on the lateral surface of the condyle which appeared to be a counterpart to the shape of the new bone. The appearance of this bone was rapid, with all specimens exhibiting it on day 20 and thereafter. The Alcian stained specimens illustrated the bone as a small spot of cartilage several days before its appearance. The function of this bone has not been determined.

Baculum

The baculum is a heterotopic bone of the penis, found in most insectivores, bats, carnivores, non-human primates and rodents (Vaughan, 1978). The baculum first appeared in one-day-old specimens and was seen in every age group thereafter (Fig. 5). At first appearance, it appears as a rod-like structure having no other characteristics. By day 28 it appears as a rod-like structure with an enlarged and rounded distal end. No grooves could be detected.

In general, Table 1 illustrates that those elements of the appendicular skeleton ossify and mature at a faster rate than those of the axial skeleton. This is not unexpected as early neonatal locomotor patterns are necessary for nest and suckling movements. In addition, grasshopper mice leave the nest around 15-20 days of age (Bailey and Sperry, 1929) and the skeletal support systems for general locomotion must be in place at that time.

Initial complete ossification of centers began about day 3 and then proceeded very rapidly, with most ossification centers being in place by day 14 (23 of 30 centers studied). Day 9 represented the greatest completion of ossification, with 15 of the 30 centers being completed. These results are similar to that found by Kirkland (1970) who studied two species of *Peromyscus* and showed ossification completed by day 14. Finally, because of the large degree of individual variation in ossification, we do not feel that postnatal ossification in the grasshopper mouse lends itself to age determination.

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