



The degree of ossification in a chronological series of ground squirrel embryos
by Betty A Wernli

A THESIS Submitted to the Graduate Committee in partial fulfillment of the requirements for the
degree of Master of Science in Zoology
Montana State University
© Copyright by Betty A Wernli (1939)

Abstract:

The chronological series of embryos were prepared according to Lundvall's modification of the van
Wijhe staining method with Shultze's well-known KOH clearing procedure.

The method used rendered the embryos transparent with the exception of the skeleton system. The
chondroid tissue retained the toluidin blue stain. The ossified tissue was distinguished by its granulated
appearance.

Nine consecutive stages of the embryos were studied to determine the cartilage dissolution and bone
replacement by the appearance and extent of ossification centers.

As the size of the embryos increased, more ossification centers were present and their growth was
noted# The order of appearance of ossification centers was determined# The youngest embryo studied
showed no ossification center whatsoever# The cartilaginous skeleton was well blocked out.
Membranous structures were present# The half centres had not fused nor had the sternal cartilages *
The largest specimen studied had nearly completed gestation# The skeleton was well developed though
considerable cartilage remained# These two embryos with seven in between stages were described to
show the successive states of development of the embryonic skeletal system. The degree of ossification
of single structures for each stage of the series was found.

THE DEGREE OF OSSIFICATION IN A CHRONOLOGICAL
SERIES OF GROUND SQUIRREL EMBRYOS

by

BETTY A. WERNLI

A THESIS

Submitted to the Graduate Committee

in

partial fulfillment of the requirements

for the degree of

Master of Science in Zoology

at

Montana State College

Approved:

M. H. Spaulding
In Charge of Major Work

M. H. Spaulding
Chairman, Examining Committee

J. B. Hester
Chairman, Graduate Committee

Bozeman, Montana
June, 1939

N378
W49d

TABLE OF CONTENTS

	Page
Abstract.....	3
Introduction.....	4
Procedure.....	5
Historical.....	5
Method Used.....	5
Variations of Method.....	7
Description and Discussion of the Specimens.....	8
1.50 cm. specimen.....	10
1.80 cm. specimen.....	10
2.00 cm. specimen.....	11
2.50 cm. specimen.....	12
3.00 cm. specimen.....	13
4.00 cm. specimen.....	14
4.25 cm. specimen.....	15
4.50 cm. specimen.....	16
6.00 cm. specimen.....	17
Table I.....	19
General Discussion.....	20
Summary and Conclusions.....	22
Bibliography.....	24
Description of Plates.....	27

D 1539 Sift

ABSTRACT

The chronological series of embryos were prepared according to Lundvall's modification of the van Wijhe staining method with Shultze's well-known KOH clearing procedure.

The method used rendered the embryos transparent with the exception of the skeleton system. The chondroid tissue retained the toluidin blue stain. The ossified tissue was distinguished by its granulated appearance.

Nine consecutive stages of the embryos were studied to determine the cartilage dissolution and bone replacement by the appearance and extent of ossification centers.

As the size of the embryos increased, more ossification centers were present and their growth was noted. The order of appearance of ossification centers was determined.

The youngest embryo studied showed no ossification center whatsoever. The cartilaginous skeleton was well blocked out. Membranous structures were present. The half centros had not fused nor had the sternal cartilages.

The largest specimen studied had nearly completed gestation. The skeleton was well developed though considerable cartilage remained.

These two embryos with seven in between stages were described to show the successive states of development of the embryonic skeletal system. The degree of ossification of single structures for each stage of the series was found.

THE DEGREE OF OSSIFICATION IN A CHRONOLOGICAL

SERIES OF GROUND SQUIRREL EMBRYOS

INTRODUCTION

The problem of the complete development of the embryonic bony skeleton of the ground squirrel, Citellus richardsonii (Sab.), has received no attention by research workers. Charles H. Miller (13) has demonstrated the cartilaginous skeleton of mammalian fetuses. Alden B. Dawson (4) has stained skeletons of cleared specimens with alizarin Red S. Gordon W. Richmond and Leslie Bennett (16) cleared and stained embryos for ossification demonstration. R. W. Cumley, J. F. Crow and A. B. Griffin (3) made bone demonstration of embryos also by using alizarin Red S. Despite the fact that considerable library research was done, no references were discovered on the degree of ossification of the skeletons in a chronological series of embryos.

The desirability of this problem was increased by the abundance of Citellus richardsonii embryos* of all ages. Also, in the past few years there has appeared in the literature, several different technics in regard to clearing and staining skeletons. None of these included a report of any attempt to stain the cartilage and to counterstain the bone. Thus it was necessary to combine procedures in order to prepare the material for study.

*The fetuses used were the property of Professor Milo Herrick Spaulding, Head of the Zoology department, at Montana State College. Much credit is due him for his many helpful suggestions in getting the material in shape for study. Also, appreciation is extended to Professor C. A. Tryon, who gave valuable criticism and suggestions about this write-up.

PROCEDURE

The chronological series of embryos were prepared according to Lundvall's modification of the van Wijhe staining method. Shultze's well-known KOH clearing procedure was used.

In 1902, van Wijhe (13) demonstrated complete skeletons of small embryos which had been stained in anilin dyes, especially methylene blue. He used xylol to render them transparent and Canada balsam for a mounting medium. His success was based on the fact that cartilage takes the stain intensely and retains it after the color has been extracted from all other tissue by acid alcohol.

Two years later, Lundvall (13) introduced some improvements of the van Wijhe method making it more adaptable to larger objects. He used toluidin blue instead of methylene blue for staining purposes. Benzol replaced xylol as a clearing reagent from which the embryos were transferred to carbon bisulphide instead of Canada balsam for mounting.

Charles A. Miller (13) found through experimentation that satisfactory results could be obtained by combining Lundvall's stain with Shultze's 3 per cent KOH clearing method.

In order to stain the ossified tissue, the Gloria Hollister bone staining method (9) was used. Hollister obtained good results by adding alizarin Red S stain to a KOH clearing solution. Thus the clearing and the bone staining processes went on simultaneously.

The procedure used on the embryos in this case consists of the following steps:

The large embryos were eviscerated through a median abdominal

incision. The brain was also removed in the larger embryos through the median frontal and parietal sutures of the skull. This step was necessary because the brain and viscera do not clear in KOH solution.

Partial dehydration took place in a graduated series of alcohol solutions. The concentration of alcohol in the embryo tissues equalized the 70 per cent alcohol of the cartilaginous stain. This stain was made according to Lundvall (1 gram Grubler's toluidin blue, 100 cc. 70 per cent alcohol and 1 cc. concentrated HCl). A week was required for the toluidin blue stain to permeate the skeleton.

The embryos were then destained in acid alcohol (0.5 cc. HCl to 100 cc. 70 per cent alcohol). This treatment proceeded until the color was dislodged from all tissues except the cartilage. This point was indicated by a blue-grey color of the embryos. A lighter color is not desirable because some destaining occurs during clearing.

Dehydration took place in 80 per cent and 95 per cent alcohol before the embryos were transferred to a 2 per cent KOH clearing solution to which had been added 0.1 cc. alizarin Red S to each 100 cc. KOH. The Gloria Hollister alizarin Red S stain consists of 1 cc. saturated solution of alizarin Red S, 5 parts of 50 per cent acetic acid, 10 parts glycerin, and 60 parts of 1 per cent chloral hydrate.

When the embryos had become quite clear, they were transferred to 20, 40, 60, and 80 per cent glycerin in distilled water. Finally they were stored in pure glycerin to which had been added a few crystals of thymal to prevent molding. Additional clearing took place in the glycerin.

The finished specimens were transparent with the exception of the

skeletal system. The cartilage appears a deep blue. The bony tissue is easily distinguished from the chondroid material by the yellow granulated appearance.

The 3 cm. specimen was collected this spring and was immediately submerged in 95 per cent alcohol. The bulk of the material was several years old and had been fixed in Bouin's solution for several years. With the one stage for comparison, it appears that material preserved in alcohol took the stain and cleared better and more rapidly than the specimens fixed in Bouin's.

Further work could be based on the power of absorption of alizarin Red S. Hollister (8) advocates the use of alizarin Red S for fish bone staining. Ossification centers of the ground squirrel did not take the alizarin Red S as anticipated. Bony tissue could not be mistaken because of its different texture from that of the cartilage.

Variations of the method described above were tried on succeeding series of embryos.

It was found that increasing the KOH concentration of the clearing solution decreased the clearing time considerably. On the material used, a 3 per cent and a 4 per cent KOH solution worked satisfactorily with no evidence of maceration of the tissue. Schultz had advocated the use of 2 per cent KOH.

The alizarin Red S in the KOH had a tendency to precipitate and it was necessary several times to filter the solution and again add the prescribed amount of dye.

Acting on a suggestion that potassium alum was a mordant, the em-

bryos were placed in a 1 per cent solution of potassium alum before staining. The results proved to be negative. The embryos did not hold the stain as well as those which had not undergone this treatment.

It was noted that the anterior appendages took the stain better than the posterior appendages and that the older embryos held it more readily than the younger ones.

DESCRIPTION AND DISCUSSION

In the literature there are accounts of embryonic ossification.

Johnson (11) has worked out the time and order of appearance of ossification centers in the Albino mouse, one of our most important laboratory animals. Johnson stained only the bone by using alizarin Red S and cleared the specimens by Shultz's KOH method. Johnson has found that the first ossification center appears in the clavicle and that in an extremely short time twenty-five centers become apparent in the skull, long bones of the appendages, and the bones of the girdles. Johnson also has compared the degree of ossification of the Albino mouse to that of the rat done by Strong (18), Sparks and Dawson (17), and the human as studied by Mall. She found that on the whole the order of appearance of ossification centers is almost the same in the three animals. With the exception of the tibia, which ossifies late in the rat, the bones of both the anterior and posterior appendages appear in the same order in the mouse, rat, and man.

Sparks and Dawson (17) have made a detailed study of the order and time of appearance of centers of ossification in the fore and hind

limb of the Albino rat, with special reference to the possible influence of the sex factor. They found that the centers of ossification of the fore foot appear, in general, earlier, and at a more rapid rate than those of the hind foot. Incidentally, it was determined in this study that the females are in advance of the males in regard to the time of appearance of ossification centers.

The time of appearance of ossification centers and the skeletal development in the ground squirrel is somewhat different than either the rat, mouse, or man.

According to Johnson (11), the clavicle is the first bone to show an ossification center in the mouse, rat, and man. In the ground squirrel, the humerus is the first structure to show an ossification center. Also, in this animal, the clavicle never undergoes chondrification. It is composed of membranous bone.

In the case of the rat, after the appearance of the first ossification center in the clavicle, twenty-five centers become apparent in a short time in the skull, appendages, and girdle. In the ground squirrel after the appearance of an ossification center in the humerus, other centers soon appear in the scapula and appendages. However, the innominate structure shows no indication of ossification until much later.

According to Strong (18), the distal phalanges of the rat do not ossify until after birth. The distal phalanges of the ground squirrel show ossification at a comparatively early stage.

Nine stages of the series of embryos will be described. They show the progressive appearance of ossification centers and the increase in

ossification almost to the end of gestation.

Table I, page 19, is a summary of the findings of this research.

I - 1.50 CM. SPECIMEN

The 1.50 cm. specimen shows a complete cartilaginous skeleton with the exception of the membranous frontal, parietal and nasal regions, the maxillaria superiores, and most of the mandible. The frontal region appears as two separate plates on either side of the skull. The parietal region also is composed of two non-fused plates. The membranous angulare and dentale form the mandible. Meckel's cartilage could not be discerned. The upper and lower jaws are widely separated and their halves have not completely symphysized.

The vertebral column is well blocked out in cartilage and appears as two rows of vertebral halves beneath the neural tube.

The cartilaginous ribs are in place.

The sternum appears as a pair of longitudinal cartilages.

The clavicle cannot be distinguished. Its presence may be hidden by the mandible.

The bones of the appendages are blocked out in cartilage. The hands and feet appear as paddles.

II - 1.80 CM. SPECIMEN

The 1.80 cm. specimen shows skeletal development in the following respects:

The frontal plates of the skull have grown closer together but are not fused. The occipital cartilaginous plates are divided. Meckel's

cartilage appears as a core in the mandible and is connected with the ear proximally. Distally the cartilage cores have not fused. The mouth has decreased in size and has begun to assume the normal crescentic shape.

The space between the half centros has decreased. The vertebral arches are still undeveloped.

The paired cartilages of the sternum are more closely approximated though no fusion has occurred.

Gegenbauer's theory (8) of the covering bone formation of the clavicle has been borne out in this work. The clavicle is the only bone in the whole skeleton with the exception of the membranous skull bones that does not go through the chondrification process. It is composed of membrane bone.

An ossification center is present in the middle of the shaft of the humerus. No other signs of ossification centers are evident in this specimen.

Marginal indications of the digits have made their appearance.

III - 2.00 CM. SPECIMEN

The 2.00 cm. specimen shows the following advancements over the younger stages:

The membranous bones of the skull have increased in size. Only a line of division between the frontal plates exists in this specimen. The cartilaginous occipital plates are completely fused. The upper and lower jaws are united at the point of symphysis.

The half centros are not fused. The vertebral arches show some

development.

A small ossification center is located proximal to the neck of the scapula.

Ossification centers are apparent in each rib, distal from the neck.

No fusion has occurred in the paired cartilages of the sternum.

The ossification center of the humerus has increased in size distal to the deltoid crest.

Ossification centers in the radius and ulna have made their appearance.

Centers of ossification have appeared in the middle of the femur and in the middle of the tibia and fibula.

The digits are more pronounced than in the former specimen.

IV - 2.50 CM. SPECIMEN

The 2.50 cm. specimen shows progressive development over the preceding embryos in the following respects:

The frontal plates of the cranium are completely fused.

The half centros of the vertebrae have begun to fuse although the vertebral arches are still incomplete.

No fusion of the sternal cartilages has occurred.

Ossification of the ribs has progressed laterally.

Ossification of the humerus is progressing.

The centers of ossification of the radius and ulna has increased.

No indication of ossification of the innominate bone is present.

The ossification center of the femur has enlarged.

A greater amount of bone deposition has occurred in the tibia and fibula than in the last described specimen.

The digits of the anterior appendages are more developed than those of the posterior appendages, although in neither is the separation complete.

V - 3.00 CM. SPECIMEN

The 3.00 cm. specimen has developed further than the younger specimens in several striking respects.

The membranous bones of the skull have grown. Meckel's cartilage has not become symphysized at the angle of the rami; cranially, it is still connected to the ear region.

The half centros are completely fused and the vertebral arches have completed the vertebral ring. The vertebrae show no indication of ossification.

Relatively each rib shows the same amount of ossification. Less than one quarter of the rib, laterally from the neck to the angle, has been ossified.

The sternal bars have fused caudally. Littermates show variation in the degree of sternal fusion. In this particular litter, one showed fusion only to the point of attachment of the third rib; in others the fusion was complete and only the xiphoid process appeared cleft.

The ossification center of the scapula is now a transverse band occupying the middle fifth of the bone.

One-fourth of the shaft of the humerus, distal to the deltoid crest, is ossified.

The radius and ulna show a larger ossified area. The carpus and manus and phalanges are completely blocked out in cartilage and the digits are separated. No ossification centers are apparent.

A beginning ossification center is apparent in the middle of the ilium. This center in the innominate is the smallest and latest to appear of all the ossification centers in the main skeleton.

The intermediate one-fifth of the femur is ossified.

A middle ossified section of the tibia and fibula includes nearly one-fifth the length.

The digits of the posterior appendages are completely separated. No ossification has occurred in the tarsus, metatarsals, or phalanges.

VI - 4.00 CM. SPECIMEN

The 4.00 cm. specimen shows increased ossification over the last described specimen.

The conversion to bone of the cartilaginous primordial cranium is noticeable in the temporal and occipital regions. The proximal end of Meckel's cartilage is not continuous with the ear. Indications of degeneration of Meckel's cartilage are apparent slightly caudad to the angle of the rami.

Indications of cartilaginous dissolution appear in the centra of the vertebrae. Definite ossification centers could not be distinguished in the vertebrae of this specimen.

More than one quarter of the rib has been ossified.

The longitudinal cartilages of the sternum have fused with the exception of the xiphoid process.

Segmentation into definite sternebrae has occurred.

Slightly less than one quarter of the scapula has been ossified.

The ossification center of the humerus has enlarged and now includes one-third the length.

Ossification centers are apparent in the middle third of the radius and ulna.

The distal phalanges have begun ossification.

With the exception of the ossified middle sixth of the ilium, the innominate structure is chondrified.

The intermediate one-fourth of the femur is ossified.

The middle one-fifth of the tibia and fibula display ossified construction.

Ossification centers are apparent in the phalanges of the hind foot.

VII - 4.25 CM. SPECIMEN

The 4.25 cm. specimen shows the following advancements over the preceding specimen:

The latero-anterior end of Meckel's cartilage has definitely undergone degeneration.

Ossification centers are apparent in the middle of the centra of the lumbar vertebrae. The spinous processes of the cervical vertebrae are ossified.

Increased cartilage dissolution appear in the lateral rib.

The longitudinal cartilages of the sternum are completely fused.

The intersternal discs of this specimen indicate cartilage dissolution.

There is no evidence of osseous tissue in this structure.

A slight increase in the ossified portion of the scapula is apparent. Ossification of this structure proceeds from the neck toward the vertebral border.

Ossification of the humerus has proceeded proximally toward the deltoid crest. More than one-third of the humerus has undergone this ossification process.

A slight increase in the amount of bony tissue in the femur, tibia, and fibula has taken place.

VIII - 4.50 CM. SPECIMEN

The 4.50 cm. specimen has attained the following advancements not present in the younger stages:

Meckel's cartilage shows increased retrogression. This process is proceeding cranially.

The thoracic vertebrae show ossification beginnings in the centra. The lumbar vertebrae show increased bone deposition.

Approximately one-third of the rib is bony tissue.

Ossification centers are present in the sternum.

The scapula shows a larger ossified strip than in the former specimen.

The ossified area of the humerus has increased to approximately two-fifths of its length. More than one-third of the radius and ulna is composed of osseous tissue.

IX - 6.00 CM. SPECIMEN

The 6.00 cm. specimen shows bony tissue which is not present in the younger stages.

The bony membranous frontal and parietal regions have grown dorsally. The covering bones of the mandible, dorsal to Meckel's cartilage has retrograded. The rami have undergone symphysis. Meckel's cartilage at this point has not degenerated. A greater amount of ossification has occurred in the occipital region than in the last described specimen.

The greatest portion of the centra of the vertebrae, from the cervical to the sacral inclusive show bone deposition. The articular processes of the cervical vertebrae, in addition to the spinous processes, have been ossified.

About one-half of each rib has undergone bony replacement.

The intersternal discs have increased in bony tissue.

Ossification of one-half the scapula has progressed toward both the glenoid cavity and the vertebral border.

Osseous deposition has progressed both proximally and distally in the humerus. Less than one-half this structure has remained chondrified.

A marked increase in the amount of osseous material in the radius and ulna has taken place. Approximately two-thirds of each structure is ossified.

Increased ossification has occurred in the ilium.

More than one-third of the femur has been replaced by bony tissue and slightly more than one-third the tibia and fibula.

TABLE I

TIME OF APPEARANCE OF OSSIFICATION CENTER

SPECIMEN SIZE	VERTEBRAL COLUMN	STERNUM	RIBS	SCAPULA	HUMERUS	RADIUS ULNA	ARMS	INNOMINATE	FEMUR	TIBIA FIBULA	POSTERIOR LEGS
1.5 cm.	Centras halved - arches undeveloped cartilage	Cartilaginous paired sternal bars	cartilage	cartilage	cartilage	cartilage	Paddle shaped	cartilage	cartilage	cartilage	paddle shaped
1.8	inter-centra space decreasing	inter-sternal space decreasing			small center of ossification present		begin separation				begin separation
2 cm.			ossification center present	ossification center present	increased ossification	ossification center present			ossification center present	ossification center present	
2.5 cm.	half centras have begun fusion		ossification increased		ossification progressing	increased ossification	developing		ossification progressing	ossification progressing	developing
3 cm.	half centras fused - vertebral ring complete	fused caudally	1/4 ossified	1/5 ossified	1/4 ossified		digits completely separated	ossification center present	1/5 ossified	1/5 ossified	digits completely separated
4 cm.	cartilage dissolution	fused except xiphoid process	1/4 ossified	1/4 ossified	1/3 ossified	1/3 ossified	ossification center in phalanges	1/6 ilium ossified	1/4 ossified		ossification center in phalanges
4.25 cm.	ossification of centra in lumbar vertebrae	bars completely fused									
4.5 cm.	centra of thoracic vertebrae ossified	ossification centers present	1/3 ossified		2/5 ossified						
6 cm.	articular & spinous processes ossified	ossification of intersternal discs	1/2 ossified	1/2 ossified	1/2 ossified	1/3 ossified			1/3 ossified	1/3 ossified	

GENERAL DISCUSSION

In this research the writer has found that the ossification and developmental processes of the skeleton of Citellus richardsonii vary from the processes in the rat, mouse, and man.

As mentioned earlier in this article, the first ossification center in the embryo of Citellus richardsonii appears in the humerus. In a little later stage ossification centers are present in the ribs, scapula, radius and ulna, femur and tibia and fibula. An ossification center is not present in the pelvic girdle until the 3 cm. stage. Ossification centers are apparent in the distal phalanges, also in the 3 cm. stage.

The clavicle in Citellus richardsonii never becomes chondrified. It is composed of bony membrane.

Johnson (11) has found that the first ossification center appears in the clavicle and that in an extremely short time twenty-five centers appear in the skull, long bones of the appendages and the bones of the girdles. Johnson found by comparison that the ossification of the main skeleton of the Albino mouse, the rat, as investigated by Strong (18), Sparks and Dawson (17) and the human showed the same order of ossification centers. With the exception of the tibia, which ossifies late in the rat, the bones of both the anterior and posterior appendages appear in the same order in the mouse, rat and man.

According to Strong (18) the distal phalange of the rat does not ossify until after birth. It thus becomes apparent that the ossification process proceeds differently in the ground squirrel, rat, mouse and man.

The distal phalanges of Citellus richardsonii show ossification

centers at a comparatively early fetal stage. In contrast, Strong (18) has found that ossification centers are not apparent in the rat until after birth.

In the ground squirrel, the clavicle composed of bony membrane and consequently never shows a cartilaginous constituency. In the man, rat and mouse, the clavicle is the first cartilaginous structure to show ossification. In the ground squirrel, the humerus is the first bone to show ossification.

In the man, rat, and mouse after the first appearance of an ossification center in the clavicle, twenty-five centers soon appear in the skull, girdle and appendages.

In the ground squirrel, the innominate structure shows no ossification until after ossification centers have appeared in the appendages and the pectoral girdle.

SUMMARY AND CONCLUSIONS

The order of appearance of ossification centers in Citellus richardsonii was determined for the main skeleton.

The skeleton is first blocked out in cartilage with the exception of the membranous clavicle, mandible (except Meckel's cartilage), brain capsule and the face, and maxillaria superiores structures. The chondroid material gradually dissolves and a bony substance is secreted in its place. This process is gradual and the changes were noted in the progressive series of embryos. Ossification centers begin on the edge of the middle of a bone and gradually increase to include a complete section of bone.

The first center of ossification was noticed in the humerus of the 1.80 cm. specimen. The 2.00 cm. specimen showed small ossification centers in the ribs, scapula, radius and ulna, femur, and the tibia and fibula. An ossification center of the ilium was first observed in the 3.00 cm. specimen. The 6.00 cm. specimen (the oldest) has nearly completed gestation. The ossification process has developed since the youngest stage but considerable cartilage remains. This order of appearance of ossification centers differ from the order in the mouse, rat, and man in which the clavicle is the first to ossify and is followed in an extremely short time by ossification centers in the skull, appendages, and girdles.

The bony replacement process precedes more rapidly in the anterior skeleton than in the posterior portion, that is, ossification of the humerus begins in the 1.80 cm. specimen. Indications of ossification of the femur are first apparent in the 2.00 cm. specimen. In the 6.00 cm. specimen, one-half the humerus is ossified and only one-third the femur.

Of the main skeleton the innominate structure is the last to begin ossification. In the mouse, this structure ossifies no later than the appendages.

Littermates show variation in the degree of development of the skeleton. The variation in one litter included sternal fusion all the way caudally from the level of the third rib to the xiphoid process.

The half centras fused before the paired cartilages of the sternum. The left and right plates of the skull fused before either the half centras or the sternal cartilages.

Gegenbauer's theory (8) of the bone covering formation of the clavicle has been shown in this work. The first appearance of the clavicle is in bony membrane form. In the rat, mouse and man, the clavicle is the first chondroid structure to show an ossification center.

BIBLIOGRAPHY

1. Bateson, Oscar V.

1921---THE DIFFERENTIAL STAINING OF BONE. *Anat. Rec.*, vol. 22, no. 3, pp. 159-163.

2. Bensley, B. A.

1922---PRACTICAL ANATOMY OF THE RABBIT. 3rd ed. P. Blakiston's Son and Co., Philadelphia.

3. Cumley, R. W., Crow, J. F., & Griffin, A. B.

1939---CLEARING SPECIMEN FOR THE DEMONSTRATION OF BONE. *Stain Technology*, vol. 14, no. 1, p. 7.

4. Dawson, Alden B.

1926---A NOTE ON THE STAINING OF THE SKELETON OF CLEARED SPECIMENS WITH ALIZARIN RED S. *Stain Technology*, vol. 1, no. 4, pp. 123-124.

5. Dodds, G. S.

1929---ESSENTIALS OF HUMAN EMBRYOLOGY. John Wiley & Sons, Inc., New York.

1932---OSTEOCLASTS AND CARTILAGE REMOVAL IN ENDOCHONDRAL OSSIFICATION OF CERTAIN MAMMALS. *Amer. Jour. Anat.*, vol. 50, no. 1, pp. 97-129.

6. Harman, Mary T.

1932---A TEXTBOOK OF EMBRYOLOGY. Lea & Febiger, Philadelphia.

7. Heisler, John Clement

1907---EMBRYOLOGY FOR STUDENTS OF MEDICINE. W. B. Saunders Company.

8. Hertwig, Oscar

1892---EMBRYOLOGY OF MAN AND MAMMALS. Swan Sonnenschein & Co., London.

9. Hollister, Gloria

1935---CLEARING AND DYEING FISH BONE FOR STUDY.
Stain Technology, vol. 10, no. 1, p. 37.

10. Howell, A. Brazier

1926---ANATOMY OF THE WOOD RAT. The Williams &
Wilkins Company, Baltimore.

11. Johnson, Myra L.

1933---THE TIME AND ORDER OF APPEARANCE OF OSSIF-
ICATION CENTERS IN THE ALBINO MOUSE. Am.
Jour. Anat., vol. 52, no. 2, pp. 241-271.

12. McEwen, Robert S.

1923---VERTEBRATE EMBRYOLOGY. Henry Holt and Co.,
New York.

13. Miller, Charles H.

1921---DEMONSTRATION OF THE CARTILAGINOUS SKELETON
IN MAMMALIAN FETUSES. Anat. Rec., vol. 20,
no. 4, pp. 415-419.

14. Neal, Herbert V., & Rand, Herbert W.

1939---CHORDATE ANATOMY. P. Blakiston's Son & Co.,
Inc., Philadelphia.

15. Reighard, Jacob, & Jennings, H. S.

1901---ANATOMY OF THE CAT. 2nd ed. Henry Holt &
Co., New York.

16. Richmond, Gordon W., & Bennett, Leslie

1938---CLEARING AND STAINING OF EMBRYOS FOR DEMON-
STRATION OF OSSIFICATION. Stain Technology,
vol. 13, no. 2, pp. 77-79.

17. Spark, Charles & Dawson, Alden B.

1928---THE ORDER AND TIME OF APPEARANCE OF CENTERS
OF OSSIFICATION IN THE FORE AND HIND LIMBS
OF THE ALBINO RAT, WITH SPECIAL REFERENCE TO
THE POSSIBLE INFLUENCE OF THE SEX FACTOR.
Am. Jour. Anat., vol. 41, no. 3, pp. 411-445.

18. Strong, R. M.

1925---THE ORDER, TIME, AND RATE OF OSSIFICATION
OF THE ALBINO RAT.

Am. Jour. Anat., vol. 36, pp. 313-355.

Figure 1. [Faint text]

Figure 2. [Faint text]

Figure 3. [Faint text]

PLATE I

Figure 4. [Faint text]

Figure 5. [Faint text]

DESCRIPTION OF PLATES

PLATE I.

- Figure 1. Lateral view of 3 cm. specimen X 2.
Figure 2. Ventral view of 3 cm. specimen X 3.
Figure 3. Ventral view of 3 cm. specimen X 3.
Figure 4. Lateral view of 4 cm. specimen X 1 3/4.

PLATE II.

- Figure 1. Lateral view of 6 cm. specimen X 1 3/4.
Figure 2. Ventral view of 6 cm. specimen X 1 3/4.

PLATE I.



Fig. 1.



Fig. 2.

Fig. 3.



Fig. 4.

PLATE II.

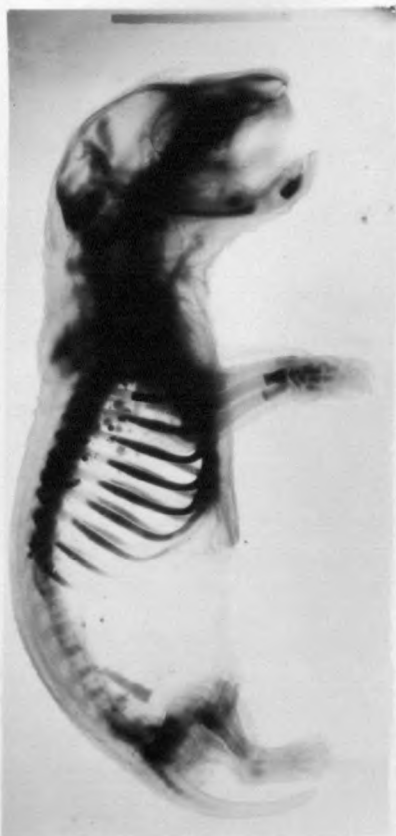


Fig. 1.

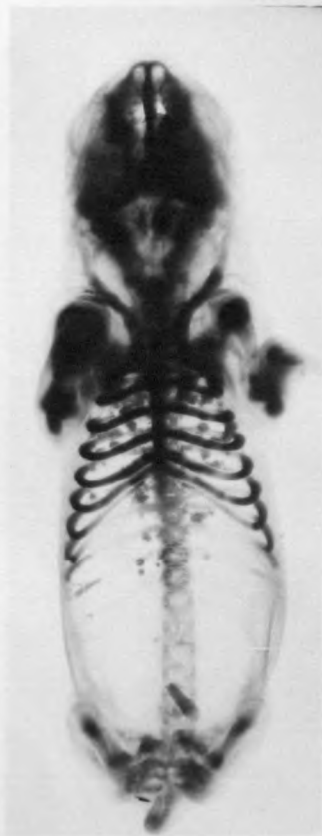


Fig. 2.

