SELECTED SKELETAL DEVELOPMENT IN MEXICAN FREETAILED BATS,

Tadarida brasiliensis mexicana (Saussure)

By

GORDON EDWARD BECKETT

Bachelor of Arts

Bethany Nazarene College

1957

Submitted to the faculty of the Graduate School of
the Oklahoma State University
in partial fulfillment of the requirements
for the degree of
MASTER OF SCIENCE
May, 1963

SELECTED SKELETAL DEVELOPMENT IN MEXICAN FREETAILED BATS,

Tadarida brasiliensis mexicana (Saussure)

Thesis Approved:

Bryan P. Glass
Thesis Adviser

W. H. Irwin

Roy D. [illegible]

Lowen Mandel?
Dean of the Graduate School

ACKNOWLEDGMENTS

I wish to express sincere appreciation to Dr. Bryan P. Glass, who gave freely of his wide and varied experience while directing this study. I am most grateful also to Drs. W. H. Irwin and Donald D. Dwyer, who served on my graduate committee.

The U. S. Fish and Wildlife Service, Bureau of Sport Fisheries and Wildlife, Bird and Mammals Laboratories, provided bands and recorded banding data. Funds were provided by National Institutes of Health research grant number 9212.

I am also grateful to Mr. Alfred E. Perry who assisted in banding and collecting specimens; to my wife, Carole Lynn, for the many hours spent in typing the manuscript.

Acknowledgments are due to Mr. James Selman, on whose property the cave was located; Mr. Alvin Eddings, Superintendent of Alabaster Caverns State Park and his wife, for their many courtesies; the Oklahoma Planning and Resources Board for providing living quarters, near the cave; Messrs. Elzie Noblitt, Harold Kamas and James Selman who made field trips and helped in locating additional caves.

TABLE OF CONTENTS

Chapter		Page
I.	INTRODUCTION	1
	Remarks on the Bat Studied	2
	Description of the Cave	2
II.	METHODS AND PROCEDURES	4
	Field Methods	4
	Laboratory Methods and Materials	6
	Preparation and fixative	7
	Cartilage staining	7
	Maceration	8
	Bone staining	8
	Clearing and storage	9
III.	RESULTS	10
	Skull	10
	Sternum	14
	Ribs	16
	Forelimb	17
	Humerus	17
	Forearm	20
	Manus	22
	Carpals	24
	Pollex	25
	Metacarpals	25
	Phalanges	26
	Pelvis	27
	General Development	30
IV.	DISCUSSION	34
V.	SUMMARY	36
LITERATURE CITED		37

LIST OF FIGURES

Figure		Page
1.	Ventral Aspect of the Skull	12
2.	Dorsal Aspect of the Skull	13
3.	Ventral Aspect of the Sternum and Ribs	15
4.	Lateral Aspect of the Humerus and its Articulation with the Proximal End of the Radius	18
5.	The Dorsal Aspect of the Hand	21
6.	Ventral and Lateral Aspects of the Pelvis	28
7.	Body Weights	31
8.	Radius Lengths	32
9.	Summary Bar Graph	35

INTRODUCTION

A study of populations of the Mexican freetailed bat, *Tadarida brasiliensis mexicana* (Saussure), in certain caves in western Oklahoma was begun during the summer of 1962. The principal objective was to develop a method of determining the age of individual bats without resorting to the use of bands. Availability of large numbers of very young bats of precisely-known age made possible the corollary study, of which this is a report. This study related to the growth rate and progressive ossification of the bony skeleton and correlation of these rates with chronological age.

Field observations began the first week of June and continued until the disappearance of all bats from the area by migration about November 1. Observations were obtained during the periods of daily contact with the animals, and morphological data were obtained from individuals selected at random from within each age group.

Simpson (1960) stated there are three quantitative aspects of growth in which the zoologist may be interested. The first is the increase in some dimension of an animal over a period of time and comprises growth in its strictest sense. A change in a single dimension of an animal during a period of time is the basic measurable aspect of growth. The second aspect of growth is that of the change in the relative sizes of two dimensions in a single animal. The third aspect concerns changes in shape resulting from differential growth of the organism in different places or along different axes. The study considers all three aspects in relation to the progression of skeletal development.

While many systems could be and have been used by other authors, (Short 1961, Christian 1957), for determining the developmental status of the organism in relation to age, the skeleton has the following advantages over other systems; the changes associated with progressive maturity are readily visible in stained specimens, and occur in an orderly sequence over the entire period from birth to early adulthood. Changes are strictly morphological, and do not necessarily indicate functional achievement such as might be true of adrenals or pituitaries.

Remarks on the Bat Studied

The Mexican freetailed bat, Tadarida brasiliensis mexicana, is a member of the family Molossidae. Members of this family have the tail extending well beyond the border of the tail membrane. They all have short, dense fur and a musty odor. Miller (1907) described the general distribution of the family as being mainly in the tropical, subtropical and desert regions of both hemispheres. Burt (1952) described the range of the species as north into Washington, east into western Oklahoma and south into southern Mexico. This is the common freetailed bat of the West, where it may be found in caves or buildings, usually in large colonies. The short, velvety fur is usually Mummy Brown.[1]

Description of the Cave

Selman Cave, located on the Jim Selman Ranch, six south and seven

---

1. Captialized color term from Ridgeway (1912).

miles west of Freedom, Oklahoma, was chosen for the project. The only large entrance to the cave lies at the base of a bluff of gypsum that forms the head of a ravine. A small stream flows from the cave into Bear Creek. The entrance faces southeast and is quite large, measuring 40 feet across with an estimated height of 20 feet. The passage, about 350 feet long, extends northwest curving gradually northward for 100 feet and is roughly as wide as the entrance. Inward about 125 feet the course of the passage turns to a southwest direction and narrows considerably. The passage decreases to non-negotiable size and communicates eventually with a sink hole on the prairie behind the cave. The cave itself is actually formed by the stream cutting a V-shaped gully in a clay bed underlying gypsum.

During July the noonday sun creates a light zone only to the bend in the passage. Later in August and September, as the angle of the sun comes more from the south, the light zone extends inward until twilight exists near the rear of the cave.

During heavy rains in September of 1962, the small stream in the cave overflowed and extended high on the "V" shaped floor. The action is probably an annual event, at least during years of heavy rainfall, and is responsible for removing much of the guano which accumulates in the cave each summer.

An important factor of the cave was the low ceiling, mostly accessible either by hand or with the aid of a net. The cave mouth was accessible by pickup truck, which was important in delivering the necessary gear.

METHODS AND PROCEDURES

Field Methods

The nursery population selected for study has been under observation for several years by Dr. Bryan P. Glass. A total of 50,000 bats have been banded by him during the period from 1954 to 1959.

The original objective of banding only baby bats with the umbilical cord still attached, proved difficult. Most young were born during the daytime. To band only young bats with umbilical cords meant that banding had to be done in the daytime when the adults were also in the cave. Such activities disturbed the adults to the point that many left the cave. A search of other caves in the area revealed large populations still present. Banding operations were therefore switched to the night hours when the adults were absent from the cave on feeding flights and within a few days the adult population returned to its former abundance.

Evidently the umbilical cord drops within a few hours of birth, for after nightfall it was difficult to find newborn bats with umbilical cords still attached. For this reason the procedure was changed, and only bats with a small umbilical scab were banded. It was assumed that such bats were less than three days old, and weights and measurements taken later supported the assumption.

Bats were banded on each forearm using number two Fish and Wildlife Service bands in combination with an anodized unnumbered band of the same size.

Anodizing is a process of subjecting a metal to electrolytic action that provides a colored protective film. The colors used were red, black, green, blue or gold. The unnumbered aluminum bands were anodized by Mac's Plating Works, 2138 South West Boulevard, Tulsa, Oklahoma.

Because of the limited number of colors available it was necessary to use a color code; for example, a red band on the right wrist with the numbered band on the left might represent a particular date. The next period might be represented by a red band on the left and a numbered band on the right. This made it possible to identify a particular age group even after the young bats began to move about the cave.

To band in a fast and accurate manner it was necessary to collect the bats and remove them to the entrance of the cave where a table and Coleman lantern were located. It was also more convenient to do the banding where the air was fresh and where there was not constant exposure to myriads of ectoparasites.

Bats were first sexed and placed in separate containers. One investigator could then handle and restrain the bats while the bands were placed on the forearm by the assistant. This permitted 800 to 900 bats to be banded each night.

Five thousand newborn bats were banded using the procedure just described, and 5000 additional ones were banded with numbered bands only. After banding, all bats were returned to the place from which they had been collected.

It was assumed that a weekly collection of 20 individuals would provide a sufficient sample for study. Using the color band code, it was possible to determine on what day a group of bats would be the required age. On that day the cave would be searched for bats having the appropriate color band.

Because of the delicate and unpigmented condition of the skin, the first three collections of bats were eviscerated only and not skinned. All later collections were both skinned and eviscerated.

Before the bats were preserved, the dead body weights from an Ohaus triple-beam balance were recorded to the nearest 0.1 gram. The animals saved for anatomical investigations were preserved in a solution of one part formalin to nine parts water.

Laboratory Methods and Materials

The literature contains several techniques for staining the bones and clearing the soft tissues of embryos and small animals in toto (Batson 1921, Dawson 1926, Evans 1948, and Williams 1941). Each procedure represents a different opinion regarding the best fixative to be used. Dawson's (1926) original technique, calling for 95% alcohol, was used with success by some authors.

The specimens collected were preserved in 10% formalin, and for this reason the Williams (1941) technique was used. Williams' claim that tissue fixed in 10% formalin for at least one week can be better controlled during subsequent maceration in KOH was fully substantiated. Total maceration is rapid in alcohol-fixed tissues, but is retarded in formalin-fixed specimens.

Williams (1941) outlined a complete program for fixing, staining and clearing small vertebrate skeletons. He found that methyl salicylate, which had been used as a tissue-clearing agent since 1914, presented two disadvantages. The tissues had a tendency to turn brown over a period and they would undergo a slight amount of shrinkage. He also showed that specimens macerated in KOH do not undergo shrinkage. He felt that shrinkage in methyl salicylate was not enought to affect the relationships of skeletal parts greatly. To avoid any possible distortion of the parts the Williams technique was used for staining and glycerin for clearing. In an effort to reduce the cost of materials required for processing the large number of specimens used, tests were made using technical grades of potassium hydroxide and white glycerin. Both proved satisfactory. Technical grade isopropyl alcohol proved unsatisfactory because the tissues would not clear properly, but chemically pure isopropyl alcohol did and was less expensive than ethyl alcohol.

Preparation and fixation. The fixing process required the specimen be eviscerated and immersed in 10% formalin. This was done at the time of collection. Specimens were held in the fixative from the time of collection until preparation for staining.

Cartilage staining. Specimens for cartilage staining were washed for two hours in alcohol to which 10 drops of NH_4OH had been added for each 250 cc. of 0.5% HCl and 0.25 grams of toluidine blue O (certified by the Biological Stain Commission for use in Histology). The mixture was allowed to stand for 24 hours and was then passed through filter paper. Williams (1941) recommended the specimens be hardened and destained 72 hours in four changes of 95% alcohol. The specimens in this case required a considerably longer period of destaining before the alcohol solution remained clear. The muscle tissue absorbed the toluidine blue and retained it during the maceration stage.

Maceration. The specimens were macerated in daily changes of 2% KOH from 25 to 30 days. One gallon wide mouth pickle jars were used, but a container that will stand the heat created by the dissolving of KOH in water is recommended. Demineralized or distilled water must be used, otherwise the KOH will precipitate the mineral destroying the macerating effect. Demineralized water was obtained by passing tap water through a Barnstead Water Demineralizer, manufactured by the Barnstead Still and Sterilizer Company, Boston, Massachusetts. The treatment reduces NaCl to less than two parts per million. Williams (1941) recommended the use of ultraviolet rays to facilitate the maceration process. A sun lamp would be an effective aid when dealing with few specimens, but to expose even one group containing 20 specimens, would require several lamps, at an unjustifiable expense. Generally the muscle tissue was macerated in KOH until the toluidine blue was removed. To avoid total maceration, the specimens were transfered several times from 2% KOH solution back to 100% isopropyl for rehardening and returned to KOH for additional destaining. The specimens mentioned earlier as being damaged by technical grade isopropyl were salvaged by this process.

Bone staining. Bone staining must be completed before total maceration of the soft tissue. A saturated stock solution was prepared by adding alizarin red S. to 2% KOH. A staining solution could then be prepared fresh by adding a few drops from the stock solution to 2% KOH until it was a deep wine color. Specimens remained in the solution two days or until the osseous materials appeared well-stained. The stained skeleton at this stage was clearly visible. Williams (1941) recommended the use of 1% H_2SO_4 in 95% alcohol if the soft tissue appeared slightly stained. The problem did not arise in this study.

Clearing and storage. Because of the long period required to eliminate the toluidine blue stain from the muscle tissue and to stain the skeleton, the specimens were near total maceration. The specimens with staining solution were poured into a deep pan, spread over the bottom, and the staining solutions siphoned away to be replaced with alcohol. One hour later the specimens were sufficiently hardened to permit handling. Each bat was placed in a two-ounce specimen bottle containing half glycerine and half 2% KOH for one day. The solution was then removed and replaced by technical grade white glycerin. Clearing continued for several weeks. The final result was specimens with the soft tissues transparent, the osseous tissues deep red, and the cartilage dark blue.

RESULTS

Introductory Remarks

The study concerned selected aspects of bone development illustrating rapid development in a species. Emphasis was placed on relationships of individual bones or parts of bones with each other, the progress of ossification, and the enlargement or combining of bones. The study did not presume to include every aspect of the skeletal formation. The sternum, ribs and forelimbs were chosen because their development is directly associated with the ability to fly. The skull was studied because it might serve to indicate relative rapidity of bone development. The pelvis was selected because it is one of the few parts of the bat skeleton showing sexual dimorphism.

In treating the specimens prior to study no effort was made to render visible any tissues other than those showing an affinity for alizarin red and toluidine blue stains. The stains made possible differentiation between cartilage and bone, since the former takes toluidine blue, and the latter alizarin red. Descriptions are based upon observations of areas absorbing stains.

Skull

Terminology in the discussion of the skull follows Stromsten (1947). The descriptions are based on specimens placed with the long axis of the skull drawn perpendicular to the page (Figures 1 and 2).

When the skull is stained it appears to be formed of four transverse segments or rings. The most posterior of these rings is the occipital segment consisting of the basioccipital, the two exoccipitals and the supraoccipital, that eventually fuse to form a single bone. Anterior to the occipital is a segment formed by the basisphenoid, two alisphenoids and two parietals. The frontal segment encloses the anterior portion of the brain case and is composed of the frontals, the orbitosphenoids, and the presphenoids. In front, lies the rostral segment composed of the pterygoids, palatines, maxillae, premaxillae, lachrymals and a portion of the frontal bones.

Staining reveals all bones present and well developed at birth. Development of the ventral aspect of the basicranium is nearly completed, with only small sutures separating the basioccipital, basiphenoid and the presphenoid bones. The development of the cranium at birth is so advanced that only part of the sagittal suture is still open. The fontanelles along this suture are still quite large. Mesenchyme between the bones differentiates directly into bone at the closure of the spaces.

At birth the basioccipital is an inverted "Y" shaped bone (Figure 1). It does not come in contact with any other ossified material but is supported by non-staining tissue. On each side of the basioccipital are the large, rounded tympanic bullae, which project below the general level of the cranial floor. Posterior to the basioccipital is a small transverse bar of ossified material which is the ventral part of the atlas. This later becomes oval in shape and articulates with the occipital condyles. In the newborn bat the occipital bones surrounding the foramen magnum are separate. Between the second and third weeks these bones join and fuse to form the occipital bone.

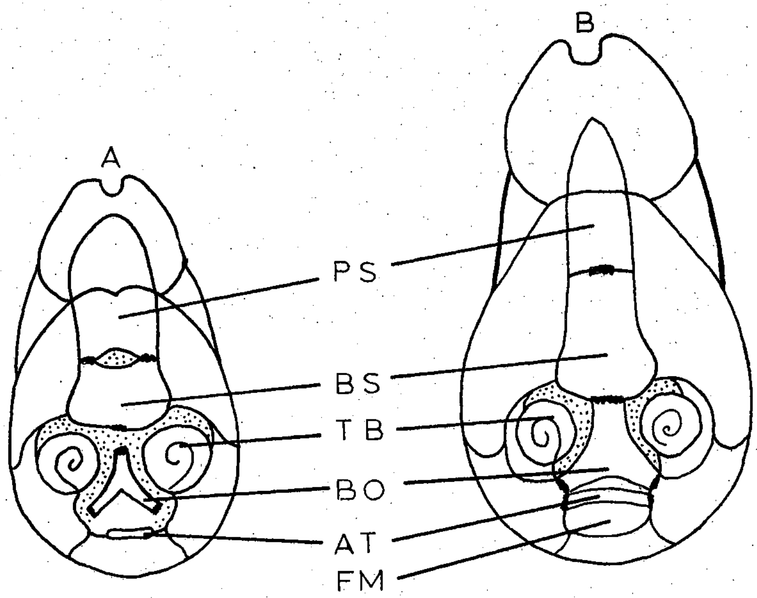

Figure 1. Ventral aspect of the skull. (A) Birth. (B) Three weeks. (AT) Atlas, (BO) Basioccipital, (BS) Basisphenoid, (FM) Foramen magnum, (PS) Presphenoid, (TB) Tympanic bulla.

Anterior to the basioccipital, but not in contact with it, is the basisphenoid, which touches the presphenoid at both of its anterolateral corners. These limited points of contact enclose a transversely elongate space (Figure 1). The posterior ends of the pterygoids extend posteriorly over each side of the basisphenoid. Anteriorly the pterygoids mark the lateral borders of the presphenoid. In the articulated skull the anterior end of the presphenoid is just behind the posterior border of the palatines.

From birth until three weeks of age, no conspicuous changes in shape occur in the bones of the basicranium except for a gradual increase in size resulting in over-all enlargement of the skull. At three weeks the skull ceases enlargement and further growth of the individual elements results in the bones coming in contact.

The dorsal aspect of the skull follows much the same developmental pattern as the ventral (Figure 2). The general outline of the skull in the newborn is smooth, oval and somewhat pear-shaped. The rostrum is flattened, short and triangular in outline. The cranium is convex and nearly circular. Three fontanelles lie along the sagittal suture. The anterior and largest is formed at the junction of the maxillae and the frontal bones. The coronal fontanelle is located at the junction of the frontal and parietal bones, and the lambdoidal fontanelle is formed by the junction of the occipital and the parietal bones. The anterior and coronal fontanelles are connected by an open segment of the sagittal suture. Specimens of the third-week age group have skulls with all sutures and fontanelles completely closed.

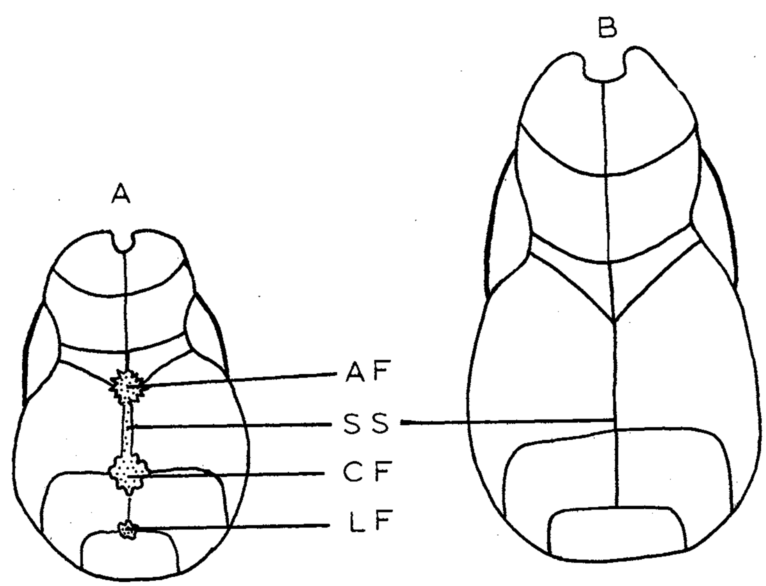

Figure 2. Dorsal aspect of the skull. (A) Birth. (B) Three weeks. (AF) Anterior fontanelle, (CF) Coronal fontanelle, (LF) Lambdoidal fontanelle, (SS) Sagittal suture.

Sternum

The sternum of the bat is composed of eight sternebrae which lie in the midline on the ventral thoraic surface and serve for the attachment of the cartilaginous portions of nine pairs of ribs (Figure 3A).

The manubrium is the anteriormost sternebra and is a "T" shaped bone at birth. The ends of the crossbar articulate with the ventral ends of the first pair of ribs. At birth this transverse portion of the manubrium is still cartilage, but the longitudinal bar is ossified except for a small portion of the posterior tip.

By the first week the manubrium has made progress in ossification and the area described as being cartilaginous at birth has been reduced. The anterior half of the transverse bar has become fan-shaped. A small area of cartilage still persists at the posterior tip of the longitudinal bar. The shape of the manubrium remains essentially unchanged throughout the second and third weeks.

Specimens of the fourth-week age group, which illustrate development between the third and fourth week, show considerable change in shape of the manubrium (Figure 3B). The most noticeable change is from a fan shape into a modified cross (Figure 3MA). The anterior and lateral points of the cross are rounded, but the posterior point is truncate. At this stage an area of previously unstained tissue near the posterior tip of the longitudinal bar appears to have differentiated into an epiphysis. This small center of ossification had been observed, but was obscure, in the third-week age group. By the fifth week this epiphysis has articulated with the epiphysis on the anterior end of sternebra number two.

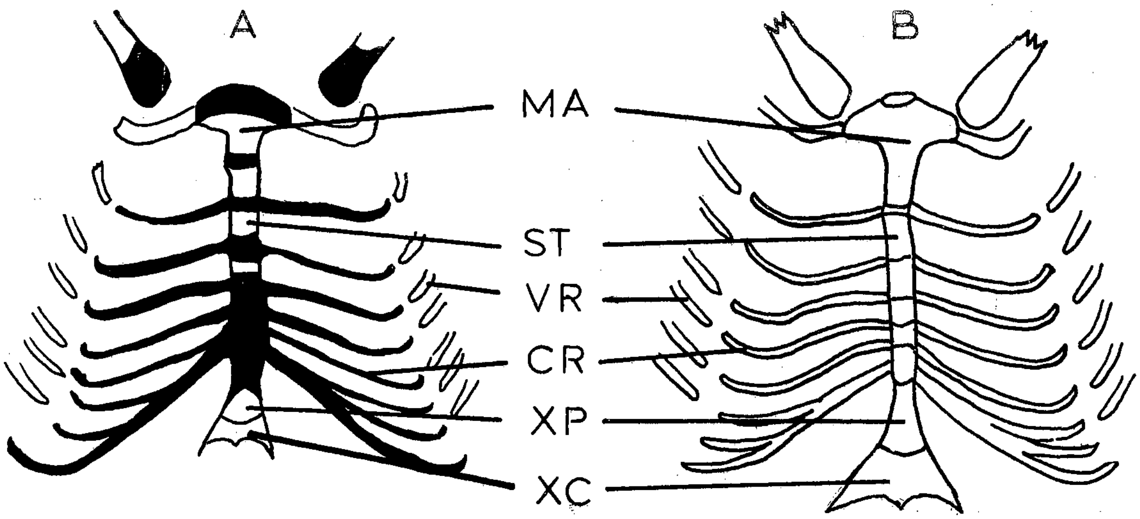

Figure 3. Ventral aspect of the sternum and ribs: (A) One week of age. (Birth not shown, as it is the same except that unossified tissue is nonstaining). (B) Four weeks. (CR) Costal portion of the ribs. (MA) Manubrium. (ST) Sternebrae. (VR) Vertebral portion of the ribs. (XC) Xiphoid cartilage. (XP) Xiphoid Process.

The remaining sternebrae form the body of the sternum, and serve as attachements for the costal portions of ribs two through nine. Comparison of specimens taken at birth indicates that these bones progress in their ossification at variable rates. In a few of the specimens the second sternebra had ossified at birth, while in others it was still cartilaginous. Conversion of cartilage begins in the anterior sternebrae and progresses posteriorly. By one week of age sternebra number two is well ossified. By the end of the second week number three and a portion of number four have ossified. By the fourth week the only remaining cartilage in the body of the sternum is a small area that serves as the attached for the Xiphoid process. This area was unstained at birth but had absorbed the toluidine blue stain in the one-week age group. The epiphyses between the manubrium and the second sternebra are the only two epiphyses in the sternum. The remaining sternebrae of the body come into contact and fuse directly. The xiphoid process develops no epiphyses.

The xiphoid process is completely ossified at birth. It stains bright red and appears to be unattached to the sternum by demonstrable skeletal tissue. At birth the shape of the xiphoid is nearly rectangular, but by the first week it has attained its characteristic elongated bell shape, which it maintains.

A flat, fanshaped piece of nonstaining material is attached to the posterior border of the xiphoid process. The structure is referred to in mammalian anatomy as the xiphoid cartilage. In the freetailed bat it does not take toluidine blue. The sternebrae forming the body of the sternum are fused by the end of the fourth week and are capable of giving substantial support at the attachement of the ribs and developing flight muscles.

Ribs

There are 13 pairs of ribs in the Mexican freetailed bat. For the convenience of description the rib proper will be referred to as the vertebral segment and the sternal portion as the costal segment (Figure 3). In ventral view the rib cage has the shape of a bell, being elongated and dorsoventrally flattened. The first rib is shorter, thicker and more strongly constructed than the other ribs, and articulates with the lateral arm of the manubrium. Ribs two through seven have individual attachments to the body of the sternum. Rib eight attaches to rib seven near the sternum and rib nine similarly attaches to rib eight. Rib number ten has the ventral costal end unattached; however, it obtains support from the tissue surrounding rib number nine.

Ribs 11, 12, and 13 are vertebral or floating ribs.

Specimens at birth have the vertebral portion of the ribs ossified, while the costal portions of the ribs will not stain. Specimens of the one-week age group show the costal portions to have absorbed toluidine blue stain. From birth until four weeks no conspicuous changes occur in the shape of the ribs, except for a gradual increase in size. The blue-staining costal portions of the ribs begin to stain red during the first and second weeks. Conversion begins with rib number one and progresses posteriorly. By the fourth week the costal, as well as the vertebral portions of the ribs are stained red. The only area staining blue is the junction of ribs seven, eight and nine. In all groups of bats studied a small area of nonstaining tissue remained at the junction between the costal and vertebral portions of each rib.

Forelimb

The following description of ossification in the forelimb includes development from birth until complete union of the phalangeal epiphyses with the shaft, which is the last stage in skeletal ossification. For convenience each segment of the limb will be discussed individually.

<u>Humerus</u>. The formation of the humerus is in three definite parts (Figure 4). The specimens at birth show the proximal epiphyses to be a nearly spherical bone about 0.8 mm in diameter, suspended in a mass of nonstaining tissue. The bone is destined to become the head of the humerus. About 2 mm distal to the head is the proximal end of the main shaft of the humerus. At birth the bone is short, stout, and round in cross section. The ossified length is about 8 mm as compared

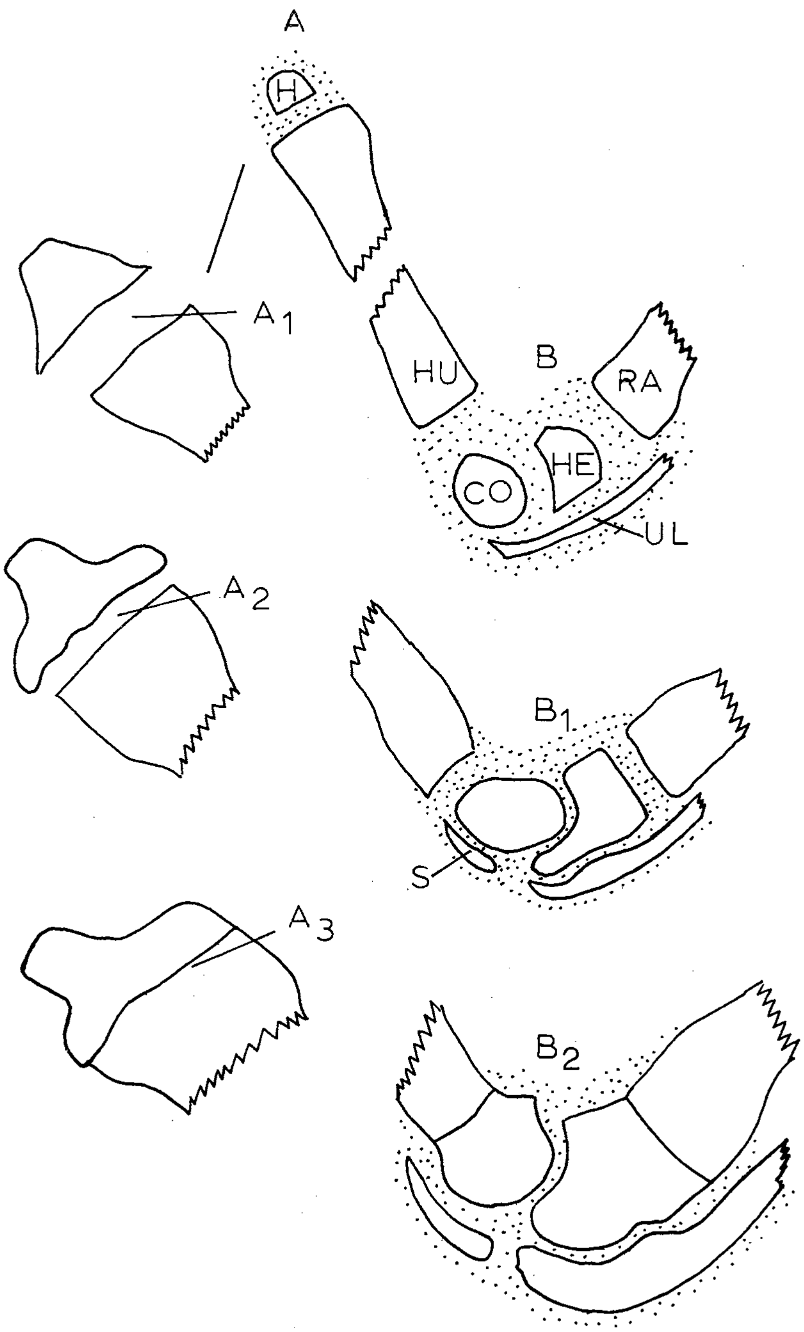

Figure 4. Lateral Aspect of the Humerus and its Articulation with the Proximal End of the Radius. (A) The head of the humerus and its relationship to the main shaft at birth. (A_1) Two weeks. (A_2) Three weeks. (A_3) Five weeks. (B) Articulation of the elbow at birth. (B_1) One week. (B_2) Three weeks. (CO) Lateral condyle. (HE) Head or proximal epiphysis of the radius. (HU) Humerus. (UL) Ulna. (S) Sesamoid bone. (RA) Radius.

to 12 mm for the radius. The internal and external condyles are spherical bones arising as individual centers separated from each other by 0.5 mm and the distal end of the shaft by 2 mm of unstaining tissue (Figures 4B). By the end of the first week the head of the humerus has changed from a subspherical to a trapezoid shape (Figure $4A_1$). It is still separated from the main shaft by about 1 mm of nonstained tissue. The main shaft has elongated, but each extremity is greater in diameter and stains darker than the middle portion of the shaft. The internal and external condyles have united to form a bean-shaped bone (Figure $4B_1$). The condyles are still separated from the main shaft by about 1 mm of nonstained tissue. In this age group a small elongated sesamoid bone, about 1 mm in length, appears at the posterior region of the condyles. It appears to be a proximal continuation of the ulna but never does fuse with it or even come in contact with it.

Specimens of the second-week age group show the head has changed from a trapezoidal to an inverted "T" shaped bone, but is still not in contact with the main shaft (Figure $4A_2$). The shaft has continued to elongate and the space separating it from the condyles has been reduced.

The third-week age group shows the head as described for the two-week group but still closer to the shaft. The shaft has elongated very little but appears to have enlarged in cross section. The distal end is in contact with the condyles; however, they do not appear to be fused. The sesamoid bone articulates with the condyles.

The fourth-week age group shows the head of the humerus to be still unattached to the main shaft. The humerus, with the exception of a bulge at the proximal end, now appears as a cylinder. The articulation at the elbow remains as described for the second-week age group.

The fifth-week age group shows the head of the humerus in contact (Figure $4A_3$) with the main shaft and articulating with the glenoid cavity of the scapula. This development unites the three centers of ossification forming the humerus.

Forearm. The radius is formed in three parts (Figures 4 and 5). At birth the shaft and a small crescent-shaped proximal epiphysis and the wedge-shaped distal epiphysis are visible. The proximal epiphysis will form the articulation of the radius with the condyles of the humerus (Figure 4B). In bats the radius is the principal bone of the forearm. The ulna does not develop a semilunar notch but articulates with the posterior surface of the head of the radius (Figure 4B).

The specimens at one week show blue-stained or unstained tissue between the distal end of the shaft and the carpals (Figure 5). In addition, the distal epiphysis has enlarged and ossified.

The proximal epiphysis of the radius has changed from a crescent to an L-shape and forms an articulation with the condyles of the humerus (Figure $4B_1$). The bottom or posterior surface articulates with the coronoid process of the ulna. The proximal epiphysis is separated from the shaft by 1 mm of nonstaining tissue. The average length of the shaft at this age is 15 mm and the diameter at the middle is 1 mm increasing to 2 mm at each extremity. Located at the distal end of the shaft is a 2 mm area of blue-stained tissue. Distal to this blue tissue is the triangular-shaped distal epiphysis (Figure 5EP). Located beside and posterior to the blue-staining tissue is a small cylindrical spicule of bone (Figure 5UL). This bone is the distal epiphysis of

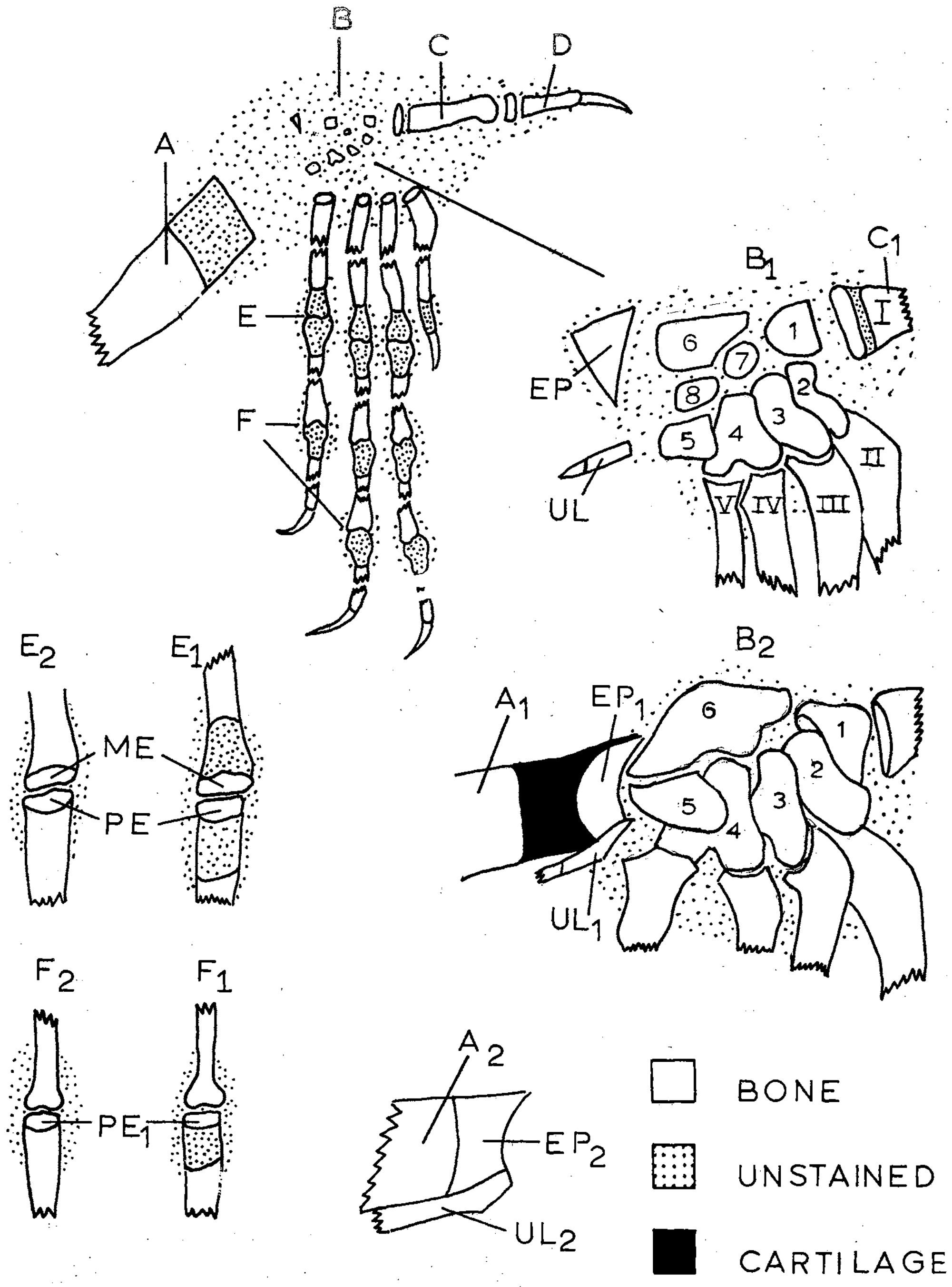

Figure 5. The dorsal aspect of the hand. (A) The distal end of the radius at birth. (A_1) Two weeks. (A_2) Five weeks. (B) Carpals at birth. (B_1) Carpals at one week. (B_2) Carpals at two weeks. (C) First Metacarpal at birth. (C_1) One week. (D) Terminal phalanx of the pollex at birth. (E) Articulation between the metacarpals and phalanges at birth. (E_1) Three weeks. (E_2) Six weeks. (EP) Epiphysis of the radius at one week. (E_1) Three weeks. (EP_2) Five weeks. (F) Articulation of the phalanges at birth. (F_1) Three weeks. (F_2) Five weeks. (UL) Ulna at one week. (UL_1) Three weeks. (UL_2) Five weeks.

the ulna, but is widely separated from the main shaft by a thread-like, nonstaining strip of tissue.

The second-week age group shows that the radius has elongated and the space separating the head and the shaft is shortened (Figure 5A). The distal one-fourth of the epiphysis of the ulna and the distal epiphysis of the radius have fused side by side. At this age the epiphysis has established a definite articulation with carpals four, five and six (Figure $5B_2$).

The third-week specimens have the head of the radius articulating with the condyles of the humerus (Figure $4B_2$). The proximal end of the ulna and the main shaft of the radius are in contact, but not fused. The distal end shows still less space separating the shaft from the epiphysis.

The fourth-week age group shows that the head of the radius has fused to the main shaft. The articulation at the elbow remains the same as in the third-week age group. The shaft has become curved with the ulna subtending the arc so formed. The space between the distal epiphysis and the shaft has been further shortened.

In the fifth-week specimens the ulna and radius are fused throughout the distal half of their length. The epiphysis of the radius and the distal end of the main shaft are in contact and there seems to be no epiphyseal cartilage separating them (Figure $5A_2$). This development completes the union of the three bones forming the radius.

<u>Manus</u>. In bats the manus consists of the carpus, composed of at least eight elements, some of which fuse; the thumb or pollex, which stands free as a separate digit, and digits two through five, which are enclosed in the flight membrane. The thumb is digit one and the fingers

are numbered consecutively two through five (Figure 5).

At birth the carpus or wrist is very weak. The pollex has ossified more than the other components of the manus, enabling the newborn to cling to a mother, or to the ceiling of the cave. The metacarpal has a well-formed proximal epiphysis which is not fused to the shaft of the bone. The first phalanx has the proximal epiphysis similarly developed, but there is no separate distal metacarpal epiphysis. Either this metacarpal has no distal epiphysis, or the two are fused prior to birth. The phalanx of the pollex has proximal and distal segments. Since this digit bears a claw, the segments may represent two distinct phalanges which are fully formed at birth except for one epiphyseal closure.

The carpus at birth shows only incipient ossification but rapidly becomes functional through the enlargement of the carpals. The eventual complement of six carpals in this species is obtained from eight original units by the union of two proximal and one medial carpal to form one massive element. The development is completed in only two weeks.

The fingers are greatly lengthened, and their metacarpals form the chief supports of the wings. They have their proximal epiphyses fused at birth but do not acquire distal epiphyses until the second week.

The phalanges develop epiphyses only at the proximal ends of their shafts. If they have distal epiphyses they are fused prior to birth. The second digit has only one phalanx. This finger is closely associated with the third digit, there being only a narrow web of skin separating them. Together they reinforce the leading edge of the wing. The third and fourth digits have three phalanges and the fifth digit has two. The terminal phalanges of digits two through five have claw-shaped tips enclosed by skin. The terminal tips appear jointed much like the terminal bone in the pollex. The terminal segment is probably only a tapering piece of calcified cartilage. There is no difference in the way the two segments take red bone stain.

Carpals. At birth the carpals consist of seven small centers of ossification in a mass of nonstaining tissue (Figure 5B). A few specimens show an eighth center of ossification. The carpals are arranged to arch distally and transversely. It was necessary, therefore, to depress the carpus and distort the natural arrangement of the bones in order to draw them on a flat plane in proper relationship to each other (Figure 5B).

The first-week age group has all of the carpals considerably larger than in the newborn. In the first-week group the eighth carpal is fully formed (Figure $5B_1$). The development gives the wrist five distal, one medial, and two proximal carpals. Carpal one articulates with the pollex distally and carpals two, six and seven proximally. Carpals two, three and four articulate with metacarpals II, III and IV respectively on the distal side. Carpal four being a "Y" shaped bone, also has a ventrolateral projection which articulates with metacarpal number V. On the distal side carpal two articulates with the proximal side of carpal one; carpal three with carpal seven, and carpal four with carpals five, seven and eight. The proximal sides of carpals one, seven and eight articulate with the distal surface of carpal six. The proximal sides of carpals five, six and eight form the articulation with the epiphyses of the radius and the ulna. In addition to these developments a small sesamoid bone develops on the palmar side, behind carpals four and five. This bone is probably comparable to the pisiform in other animals, although its location is not identical with that of the pisiform in other orders. Its position is not indicated in Figure B_1.

The second-week age group has carpals six, seven and eight united to form one massive bone (Figure $5B_2$). The other carpals show some increase in size, and are more firmly articulated with each other than in the previous week, but otherwise do not manifest any particular changes. The carpus is essentially fully formed by the time the bats are two weeks of age.

Pollex. The pollex is completely formed at birth except for the fusion of the epiphyses of the metacarpal and proximal digit, respectively. Both epiphyseal gaps close during the first week of life.

Metacarpals. Metacarpals II through V each have the proximal end and the main shaft ossified. A discernible line indicates that epiphysial closure takes place before birth. The distal end has not even formed an epiphysis (Figure 5E). Under magnification the unstained outline of the distal ends of the bones are visible, including articulation with their respective phalanges. The unossified interspaces measure about 1.8 mm and the unstained joints are surrounded by masses of jelly-like tissue. The spaces separating the phalanges also have must the same appearance (Figure 5F).

The first-week age group shows that metacarpals II through V have elongated and developed more perfect articulating surfaces that join firmly to the carpus (Figure $5B_1$). The distal articulating surfaces still resemble the condition prevailing at birth (Figure 5E).

The third and fourth-week age groups show the epiphyses to be still separated from the main shaft (Figure $5E_1$). The fifth-week specimens show the epiphyses in contact with the main shaft (Figure $5E_2$).

Phalanges. The phalanges have only the shafts and the distal ends ossified at birth (Figure 5E and 5F). The proximal articulations with the metacarpals are visible but nonstaining (Figure 5E). Digit number two, which forms the leading edge of the wing, has its only joint between the metacarpal and its single phalanx. Digits three and four have proximal, medial and distal joints. Digit number five has only a proximal and distal joint. Each joint is surrounded by a mass of nonstaining jelly-like tissue. The terminal phalanx of each digit is claw-shaped, and has a red transverse line dividing it into proximal and distal portions.

The first-week age group still has the proximal articulating surfaces of all phalanges of nonstaining tissue. A disc-shaped bone is located at the distal end of each first phalanx of digits 3, 4 and 5. The bone is the proximal epiphysis of the adjoining second phalanx (Figure $5F_1$, PE_1). The epiphysis of each first phalanx in digits three, four and five, articulates with the distal epiphysis of their respective metacarpal (Figure $5E_1$). Each epiphysis is separated from its main shaft by an area of nonstaining tissue. The joints between phalanges are distinctive in that the distal end of the phalanx is ossified and articulated with an epiphysis which is separated from its main shaft by an area of nonstaining tissue.

The third and fourth-week age groups have the articulations in the joints as described for the first-week age group. The phalanges have elongated and shortened the distance separating the epiphyses from their respective shafts.

The fifth and sixth-week age groups show the epiphyses to be in contact with the main shaft. Additional fusing of the bones occurs as the animals mature.

Pelvis. At birth the pelves of both sexes are identical. The pelvis has the anterior end of the ischium and the posterior end of the ilium joined by cartilage (Figure 6). The anterior tip of the ilium is formed of blue-staining cartilage. The superior ramus of the pubis is separated from the adjacent parts of the ilium and ischium by an area of nonstaining tissue. The space between the pubes in the midline is filled with a nonstaining tissue, which extends around the inferior ramus of each pubis and connects with the posterior end of each ischium (Figure 6A). Projecting anteriorly from the anteroventral end of the pubis is a nonstaining epipubic eminence.

The acetabulum is a deep cup-shaped depression on the lateral surface of the pelvis. At birth and at one week the developing acetabulum is completely non-staining and located entirely on the anterior end of the ischium. The ischium is separated from the other two bones by cartilage and nonstained tissue. By the second week the bones are in contact and the anterior border of the acetabulum extends over the adjoining bones (Figure 7C). In most mammals all three pelvic elements contribute to the formation of the acetabulum. In the Mexican free-tailed bat it appears to be preformed as a nonstaining cup superimposed upon the ischium only. Ossification begins at the center of the depression and progresses toward the margin of the cup, which encloses the head of the femur. The cup is further deepened by a persistent ring of nonstaining tissue attached to the bony rim.

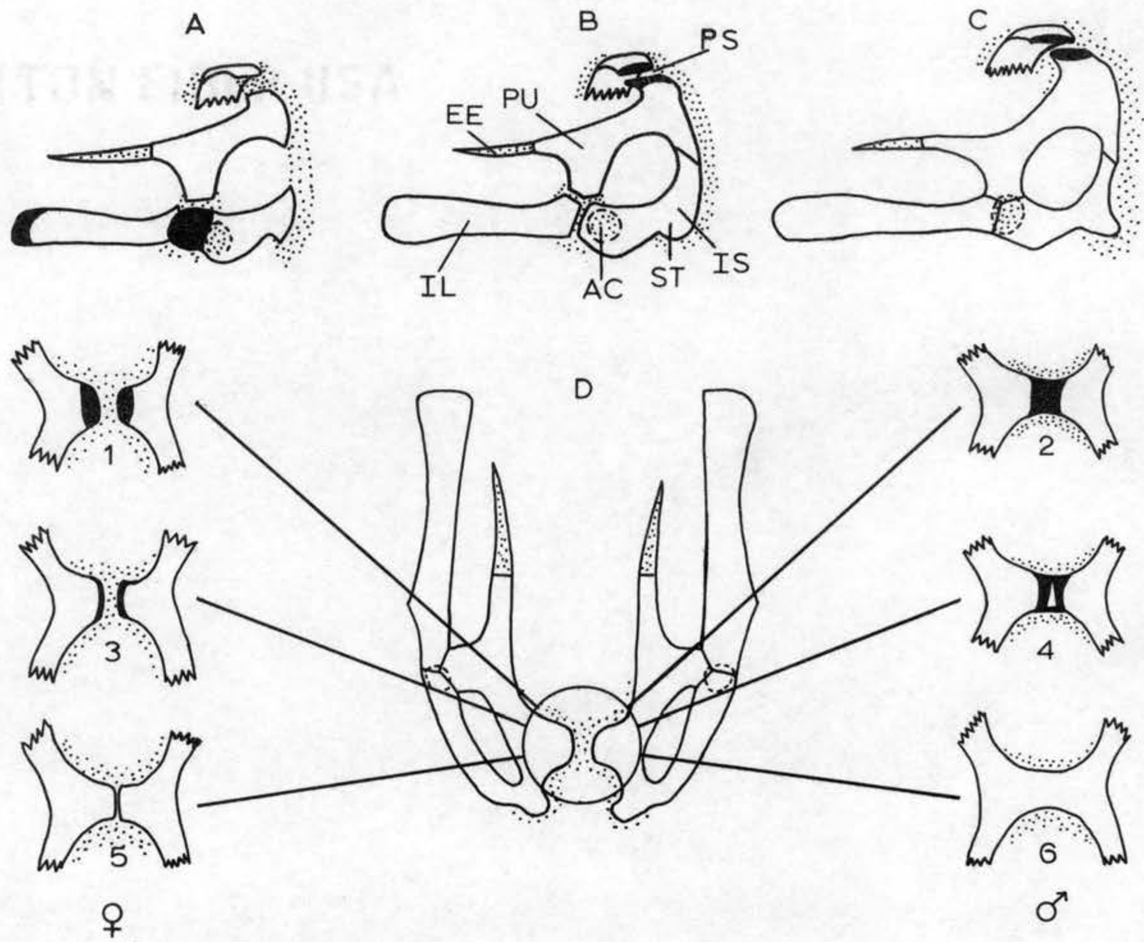

Figure 6. Ventral and Lateral Aspects of the Pelvis. (A) Birth. (B) One Week. (C) Two Weeks. (D) Ventral Aspect of the Pubic Symphysis. (D1 and 2) One Week. (D3 and 4) Two Weeks. (D5 and 6) Nine Weeks. (AC) Acetabulum. (EE) Epipubic Eminence. (IL) Ilium. (IS) Ischium. (PU) Pubis. (PS) Pubic Symphysis. (ST) Sciatic Tuberosity.

Specimens of the first-week age group show an increase in size of the whole pelvis and a reduction in the cartilage. The dorsal end of the posterior ramus of the pubis and the posterior ventral end of the ischium are in contact, thus completely enclosing the obturator foramen. A Y-shaped area of nonstaining tissue still separates the adjacent parts of the ilium, ischium and pubis.

In the first-week age group a difference between the sexes is first noted in the pubic symphysis. There is blue-staining cartilage in the pubic symphysis in both sexes. In the males the cartilage extends completely across the symphysis (Figure $6D_2$). In the females the cartilage extends toward the midline from each side but the two do not actually meet (Figure $6D_1$). The pubic symphysis in the Mexican freetailed bat differs from many other mammals, in that the processes which form the symphysis arise from the pubic bones alone, and the ischium is not involved.

The lateral view of the second-week age group shows that the three pelvic elements are in contact, under the anterior border of the acetabulum. The pelvic outline is altered posteriorly by the elongation of the sciatic tuberosity (Figure 6C). In the female the amount of cartilage in the pubic symphysis is reduced by encroachment of bone from either side, while in the males a wedge-shaped bone, surrounded by blue staining cartilage, is located in the midline (Figure 6D3 and 4).

The succeeding age groups show a gradual replacement of cartilage by bone until complete ossification of the symphysis occurs in the males of the nine-week age group (Figure 6D5 and 6). The pubic symphysis did not close in female specimens but all cartilage was replaced by bone by the end of the fifth week. Skeletons from fully adult male and female specimens in the mammal collections at Oklahoma State University have essentially the same pelvic structure as described for young bats. Since the study was not extended beyond 16 weeks, it cannot be stated positively that the pubes never fuse prior to giving birth.

General Development

The body weights recorded at the time of collection aid in establishing the rate of growth in the young population. The weights are not considered to be an accurate measurement of growth but serve to illustrate factors which are reflected in the growth curve (Figure 7). The young bats, between birth and the fifth week are entirely dependent upon the adult females for nourishment. Davis et al. (1962) referred to the community nursing system as one large dairy herd. Short (1961) stated that the response of the young to the unusual adult-to-young relationship which occurs during the fifth week, which is the weaning period, is manifested in the growth and development of the young. The weaning period is reflected in the growth curve by the loss of "baby fat" (Figure 7). Flight training at this age also contributes to the loss of weight. Bats prior to the fifth-week age group had a thick layer of white fat beneath the skin. Specimens examined after flight was initiated had a reduction in the layer of fat, and by the ninth week it was nearly absent. The young bats begin foraging flights during the sixth week and by the tenth week have regained the weight lost. Young bats in November averaged five grams below the average weights of a random collection of adults.

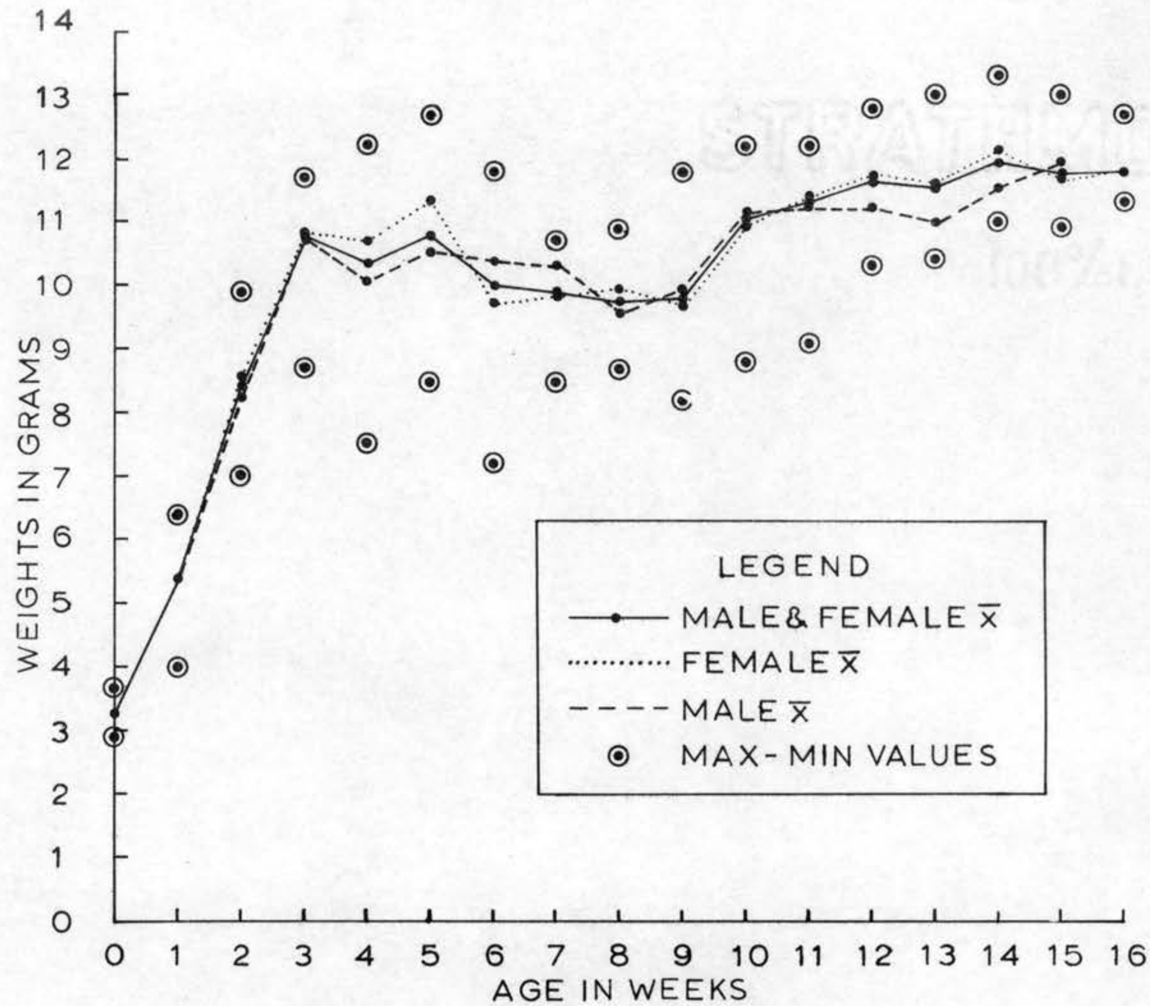

Figure 7. Body weights.

Short (1961) used the forearm as a convenient measurement of body size. He found that by the sixth week of age young bats have attained forearm lengths inseparable from those of adult animals. In this study the length of the radius, rather than the total length of the forearm, was used as an indicator of skeletal growth. The average length of the radius was used as an indicator to illustrate the rapid development made during the first five weeks (Figure 8). The variation between individuals at birth was less than 1 mm and

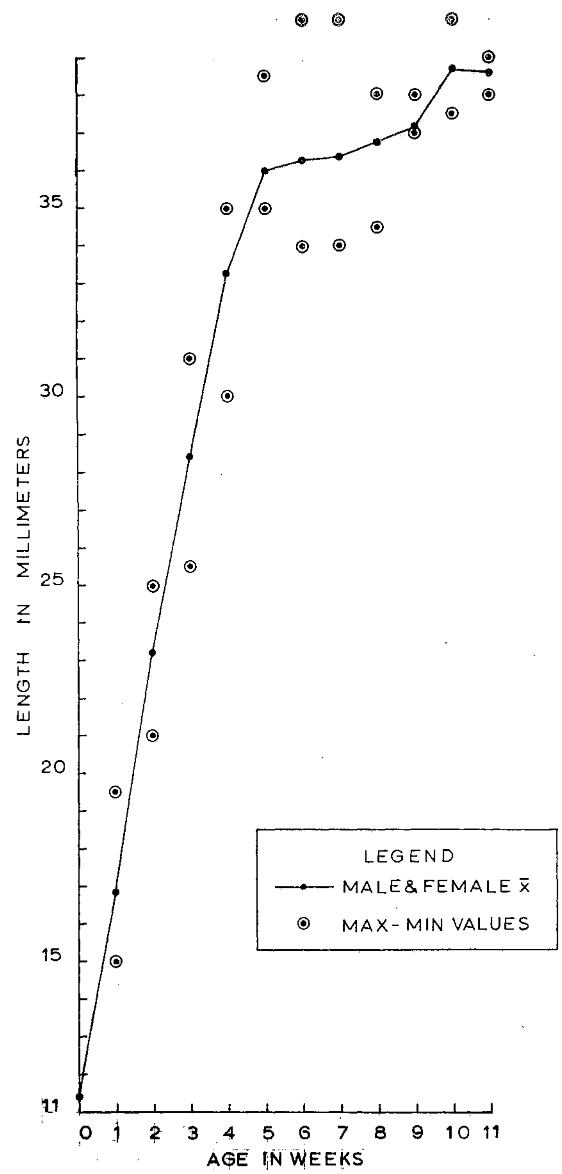

Figure 8. Radius lengths.

increased in each age group. The small amount of variation found between individuals in the earlier age groups makes it possible to make general statements concerning the advancement in development between age groups.

The specimens may be placed into proper age groups solely on the length of the radius until the fourth week. By compensating for the additional tissue in the living animal, this could be a quick method for determining the age of young bats in a nursery population.

The growth curve based on the radius length illustrates that the elongation of the radius was suppressed during the period between the fifth and ninth week, which was also the period when the specimens were in the process of being weaned and learning to fly (Figure 8).

DISCUSSION

The first observation of note from the study is that different parts of the skeleton are ossified in varying degrees when the bats are born. In mammals generally the head is proportionately further developed than other parts of the body at birth. The advanced state of development in the skull is in keeping with the nature of development in mammals generally, and reflects the comparative precocity at birth of this species. The rapid development of the thumb seems to be associated with the needs of the newborn young.

The pectorial girdle, rib cage and most parts of the forelimb are relatively undeveloped at birth. Various parts develop at different rates of speed, the most rapid being the wrist and sternum, which soon enable the young bat to crawl about in the cave. Later with development of the finger bones and the forelimb, the bat becomes capable of flying at some six weeks of age.

The pelvis seems to develop more slowly, possibly because the ability to fly is not so dependent upon the strength of the pelvis. In most of the post-cranial skeleton the rates of ossification seems to be correlated with the utilization of the various parts in flying. The rates of development are depicted as a bar graph in Figure 9, in which the developmental time for each part is depicted as a horizontal bar. The length of the abscissa corresponds to the number of weeks from birth it takes for a particular part to reach full development. The figure illustrates that the parts associated with the mechanics of flying develop much faster than the pelvis.

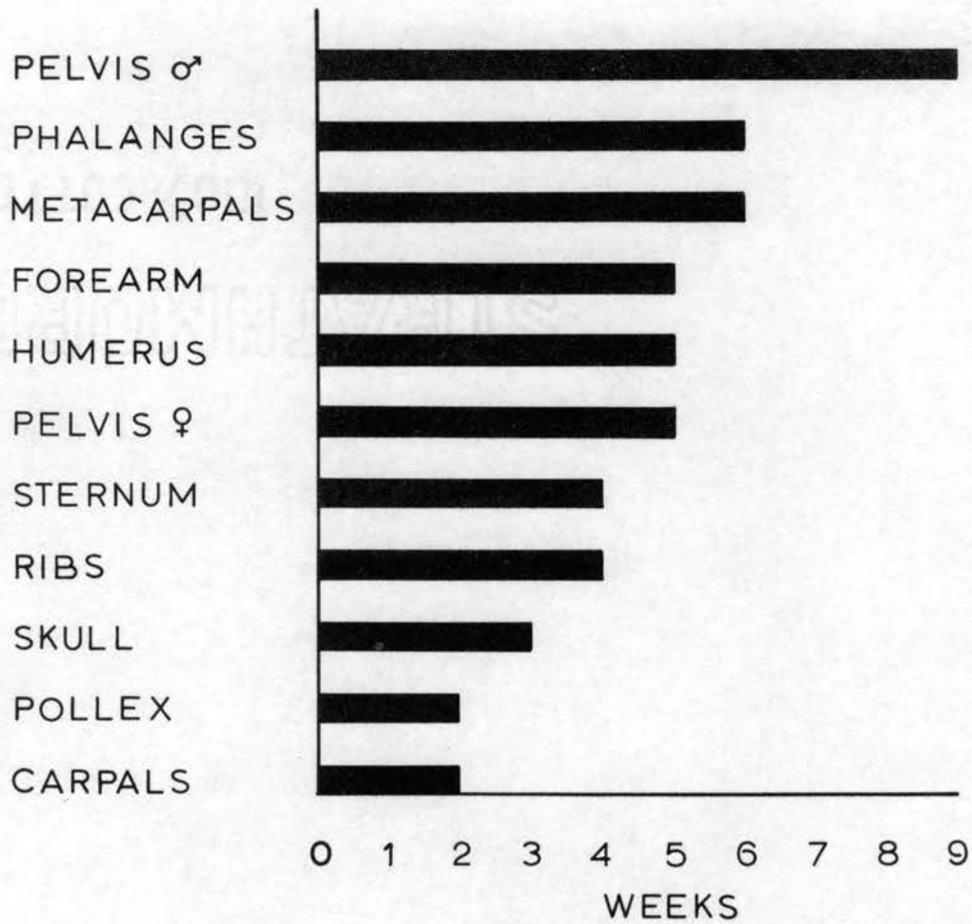

Figure 9. Summary Bar Graph. The length of the abscissa corresponds to the number of weeks from birth that it takes for that particular part to reach full development.

SUMMARY

1. A study was made of a population of Mexican freetailed bats, _Tadarida brasiliensis mexicana_, (Saussure) in certain caves in western Oklahoma. The study related to the growth rate and progressive ossification of the bony skeleton, and the correlation of these rates with chronological age. The selected areas for the study of the development covers the skull, ribs, sternum, forelimb and pelvis.
2. Specimens of known ages were successfully stained by the Williams' (1941) technique to differentiate bone and cartilage _in toto_.
3. Body weights, measurements and observations made it possible to record the rapid growth and report very little variations within the first four weeks after birth.
4. Growth and development was studied from birth until the bats were 16 weeks old, at which time they had abandoned the cave. In the study of the skeletal development it was possible to restrict most of the examination to the first five age groups.
5. The skull and pollex were nearly ossified at birth.
6. An important morphological development in connection with flight is the development of the forelimb, ribs and sternum.
7. Stained specimens revealed marked differences in the development of pubic symphysis between the sexes.

LITERATURE CITED

Batson, O. V. 1921. The differential staining of bone. Anatomy Record. 22:159-164.

Burt, W. H. and R. P. Grossenheider. 1952. A field guide to the mammals. Houghton Mifflin Company, Boston.

Cristian, J. J. and D. E. Davis. 1957. The relation of adrenal weights to body in mammals. Science, 117:78-80.

Davis, R. B., C. F. Herreid and H. L. Short. 1962. Mexican freetailed bats in Texas. Ecological Monographys. 32:311-348.

Dawson, A. B. 1926. A note on the staining of the skeleton of cleared specimens with alizarine red S. Stain Technology 14:7-11.

Evans, H. E. 1948. Clearing and staining small vertegrates in toto for demonstrating ossification. Turtox News, 26:42-47.

Miller, G. S. 1907. The families and genera of bats. Bulletin 57. U. S. National Museum. Washington, D. C.

Ridgeway, Robert. 1912. Color standards and nomenclature. Washington, D. C.

Short, H. L. 1961. Growth and development of the Mexican freetailed bats. The Southwestern Naturalist. 6:156-163.

Simpson, G. A., A. Roe and R. Lewontin. 1960. Quantitative Zoology. Harcourt, Brace and Company, New York.

Stromsten, F. A. 1947. Davison's Mammalian Anatomy. The Blakiston Company, Philadelphia.

Twente, J. W. 1956. Ecological observations on a colony of Tadarida mexicana. Journal of Mammology. 37:42-47.

Williams, T. W. 1941. Alizarin red S and toluidine blue for differentiating adult or embryonic bone and cartilage. Stain Technology. 16:23-25.

VITA

Gordon Edward Beckett

Candidate for the Degree of

Master of Science

THESIS: SELECTED SKELETAL DEVELOPMENT IN MEXICAN FREETAILED BATS, *Tadarida brasiliensis mexicana* (Saussure)

Major Field: Zoology

Biographical:

Personal Data: Born near Belleville, West Virginia, October 27, 1935, the son of Clifford F. and Rachel Mary Beckett.

Education: Attended grade school in Lowland, Nitro, and Durbin, West Virginia; graduated from Point Pleasant High School in 1953; received the Bachelor of Arts degree from Bethany Nazarene College, with a major in Biology, in May, 1957; completed requirements for the Master of Science degree in May, 1963.

Professional experience: Served in the United States Marine Corps, 1959 - 1961. March to September of 1961 as a Research Biologist for the University of Maryland, Institute of Marine Science, Solomons, Maryland.

Member of: American Society of Mammalogists, American Society of Limnology and Oceanography, The Wildlife Society, The Oklahoma Academy of Science, Phi Sigma, Society of Sigma Xi.