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The Development of the Weberian Ossicles in Pantosteus Plebius

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WEBERIAN OSSICLES - BUTLER

THE DEVELOPMENT OF THE WEBERIAN OSSICLES

IN *PANTOSTEUS PLEBIUS*

By

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INTRODUCTION

The Weberian ossicles form a chain of tiny bones that connect the air bladder and the ear of many freshwater fishes. It is thought that the ossicles are derived at least in part from the vertebrae and that they serve to transmit vibrations or changes in hydrostatic pressure to the ear from the air bladder or swim bladder.

The Weberian apparatus, which was first described by Weber in 1820, is characteristic of the largest order of freshwater fishes, the Ostariophysi, a group that includes forty-two families, among which are the minnows, suckers, siluroids, and characids. The apparatus of the family Catostomidae, the suckers, is one of the more complex because the anterior vertebrae are more highly modified than in most other groups. Although the suckers have a more complex form of apparatus, they have received comparatively little study, and this is the first developmental investigation of the Weberian ossicles of any catostomid.

The species studied, _Pantosteus plebius_ Baird and Girard, is commonly referred to as the Rio Grande mountain sucker. It is endemic to the mountain streams of the Rio Grande and Mimbres River basins (Koster, 1957, p. 46).

The Weberian apparatus in the adult _Pantosteus plebius_ consists of a movable portion, the _pars auditum_, and a support,

the *pars* *sustentaculum*.

The *pars* *auditum* (plate 1, figure 1) is composed of four ossicles and an interossicular ligament. Beginning at the anterior end and passing posteriorly, the parts of the *pars* *auditum* are: the *claustrum*, *scaphium*, interossicular ligament, *intercalarium*, and *tripus*. The *claustrum* and *scaphium* form the connection of the Weberian apparatus to the internal ear; the interossicular ligament links the *scaphium* and *intercalarium* with the *tripus*, and this, in turn, is connected to the anterior wall of the air bladder.

The *tripus*, the largest of the ossicles, is a sickle-shaped bone, whose body or main portion had three processes or rami. The articular process extends laterally from the body of the *tripus* to the centrum of the third vertebra. The medial end of this process is flared and fits into a deep groove that runs vertically across the lateral side of the centrum. The posterior process or ramus extends from the body of the *tripus* to the air bladder where it tapers into a thin, delicate extension that is embedded in the *tunica* *externa*, the outer layer of the air bladder. This slender prolongation of the posterior ramus is the transformator process. It passes through the tissue of the air bladder and curves sufficiently so that its tip projects forward out of the *tunica* *externa*. The anterior process or ramus extends forward from the body of the *tripus* and is united with the other bones of the series by the interossicular ligament.

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The *pars auditus* (plate 1, Figure A) is composed of four ossicles and an interossicular ligament. Beginning at the anterior end and passing posteriorly, the parts of the *pars auditus* are: the *claustrum*, *scaphium*, interossicular ligament, *intercalarium*, and *tripus*. The *claustrum* and *scaphium* form the connection of the Weberian apparatus to the internal ear; the interossicular ligament links the *scaphium* and *intercalarium* with the *tripus*, and this, in turn, is connected to the anterior wall of the air bladder.

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Plate I, Figure 1: Dissected Weberian Ossicles.

Plate I, Figure 2: Anterior View of the Weberian Apparatus.

Plate I, Figure 3: [illegible] View of the Weberian Apparatus.

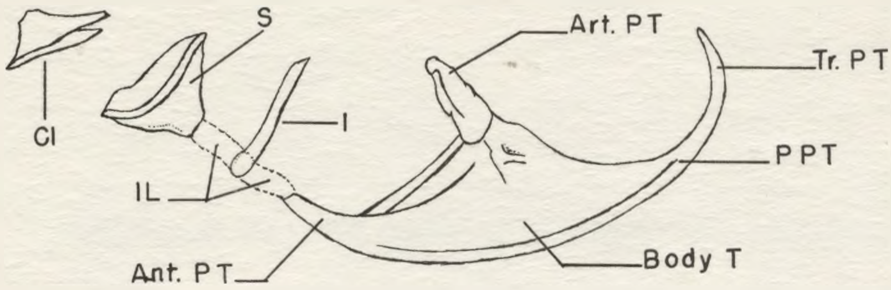

Fig. 1

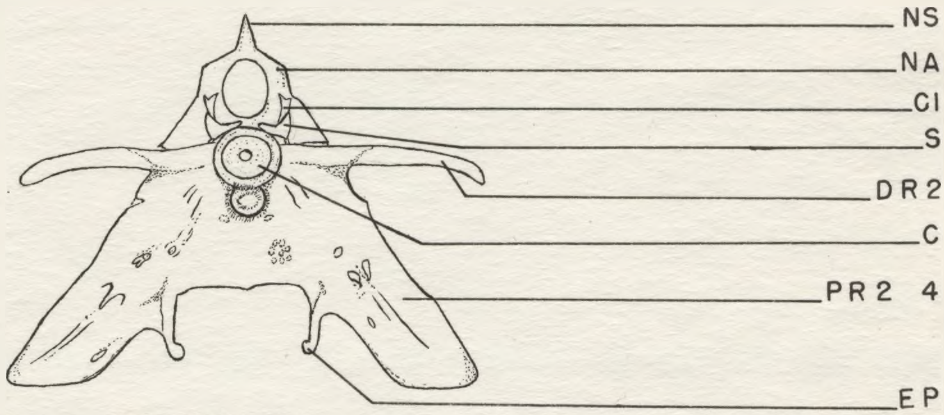

Fig. 2

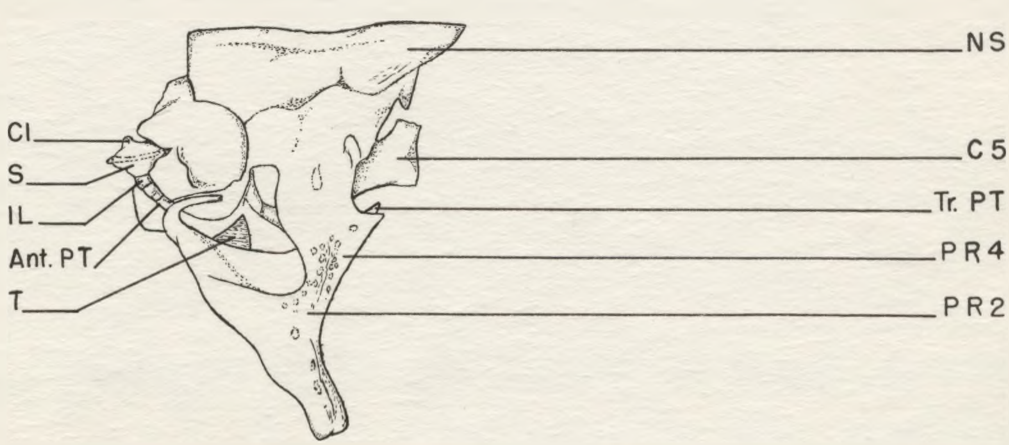

Fig. 3

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Plate II, Figure 2: Dorsal View of the Weberian Apparatus.

Plate II, Figure 3: Ventral View of the Weberian Apparatus.

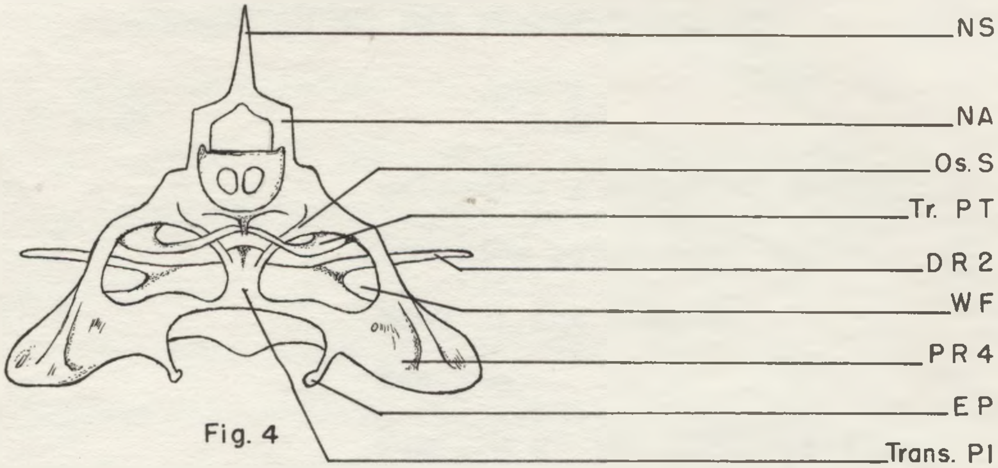

Fig. 4

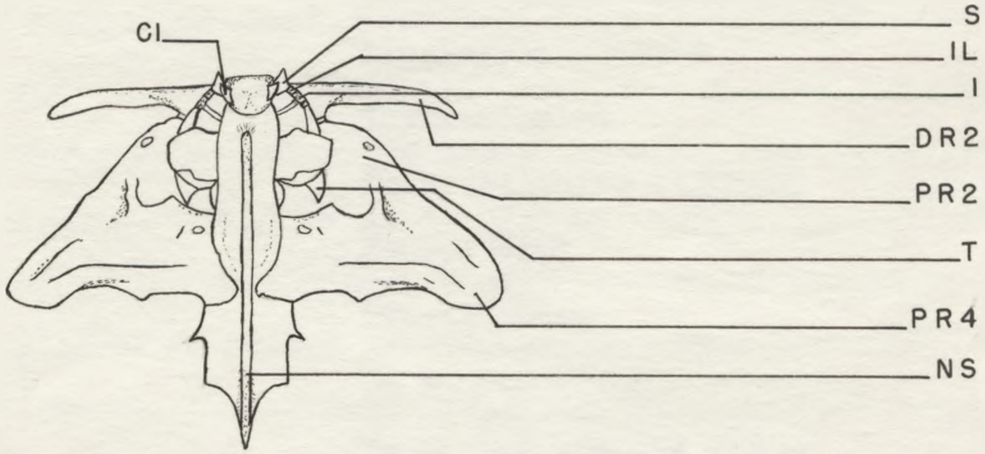

Fig. 5

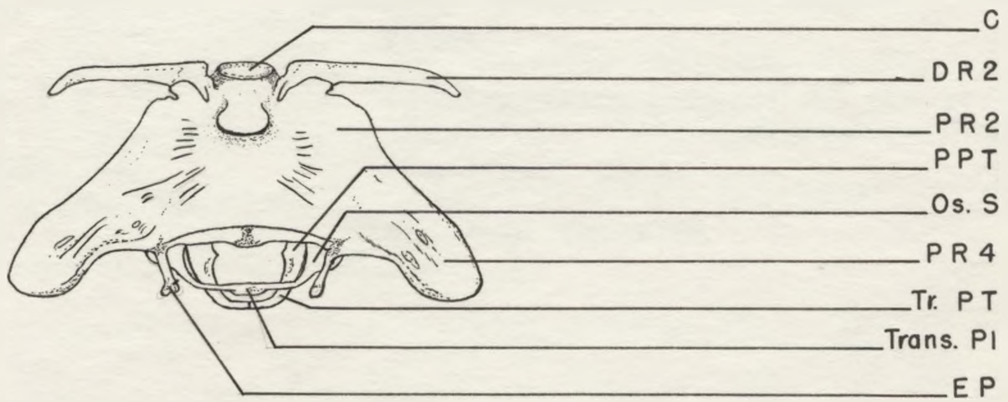

Fig. 6

ABBREVIATIONS FOR PLATES I and II

ABBREVIATION	PART
Art. P T	Articular process of the tripus
Ant. P T	Anterior process of the tripus
Body T	Body of the tripus
C	Centrum
C5	Centrum of the fifth vertebra
Cl	Claustrum
DR2	Dorsal rib of the second vertebra
EP	Esophageal process
I	Intercalarium
IL	Interossicular ligament
NA	Neural arch
NS	Neural spine
Os. S	Os suspensorium
PPT	Posterior process tripus
PR2	Pleural rib of the second vertebra
PR4	Pleural rib of the fourth vertebra
S	Scaphium
T	Tripus
Tr. P T	Transformator process of the tripus
Trans. Pl	Transverse plate
WF	Weberian fenestra

Plate I, Figure 1: Dissected Weberian Ossicles.

Plate I, Figure 2: Anterior View of the Weberian Apparatus.

Plate I, Figure 3: Lateral View of the Weberian Apparatus.

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The interossicular, or interosseous, ligament connects the anterior process of the _tripus_ to the _intercalarium_ and to the _scaphium_. In _Pantosteus plebius_ the ligament is short and is divided into two halves by the insertion of the _intercalarium_.

The _intercalarium_ is a slender, curved bone that articulates with the second vertebra and extends anteriorly and laterally to join the interossicular ligament. Two regions are distinguished: the shaft and the _manubrium incudis_, which is the enlarged lateral end that separates the interossicular ligament into two divisions.

The _scaphium_ is a cup-shaped bone bearing three processes. The cup-shaped portion is the _concha stapedis_. The first of the processes, the central process, arises from the center of the outer convexity of the cup, is aimed laterally on the fish, and is the point of attachment for the interosseous ligament. The second process is directed ventrally and articulates with the second vertebra. The third process, which points toward the head, is an extension of a ridge that runs anteriorly along the cup from the central process.

The _claustrum_ is also somewhat cup-shaped, but the medial depression is much smaller than that of the _scaphium_. The _claustrum_ lies dorsomedially to the _scaphium_ and is attached to it laterally.

The _claustrum_ and _scaphium_ plus an extension of the perilymph spaces form the connection between the Weberian ossicles and the membranous labyrinth of the ear. The

perilymph spaces, which occur between the membranous labyrinth of the internal ear and the bone of the cranium, extend posteriorly from each ear. The extensions from the two sides join and form the atrium sinus impar, which surrounds the dorsal half of the spinal cord back as far as the first vertebra. The scaphium forms a lateral part, and the claustrum a dorsolateral part, of the wall of the sinus.

The Weberian ossicles have three points of articulation with the vertebral column. These points, which act as fulcra for the movement of the ossicles, lie at the medial ends of the ventral process of the scaphium, shaft of the intercalarium, and articular process of the tripus.

The pars sustentaculum (plates I & II) includes the first four vertebrae, all of which are highly modified from the pattern of the remaining trunk vertebrae. There is much fusion of the parts. The centra of the second, third, and fourth vertebrae are coalesced. The supradorsal or neural spine elements of these three vertebrae form a large, continuous neural spine that is the most dorsal portion of the pars sustentaculum. The distal ends of the second and fourth ribs are joined.

The first vertebra is a thin disc without ribs or other projections. Only the centrum is present and it is greatly flattened antero-posteriorly.

The centrum of the second vertebra bears two processes. A transverse process, which represents a modified dorsal rib (Watson, 1939, p. 463), is directed laterally and slightly

posteriorly (plate II, figures 2 & 3), while a heavy, plate-like process, which represents a strongly modified ventral rib (Watson, 1939, p. 456), extends ventrally and caudally and fuses with the similarly modified rib of the fourth vertebra. The two ventral ribs of the second vertebra also fuse with one another beneath the centrum. The plate thus formed has a pair of posteriorly directed projections that are termed esophageal processes (plate I, figure 2) because of their proximity to the lateral walls of the esophagus.

The third vertebra lacks transverse and ventral processes. A deep, vertical groove in the lateral surface of the centrum receives the articular process of the *tripus*.

The fourth vertebra possesses a single, much enlarged rib that passes ventrally and forms, by its fusion with the ventral rib of the second vertebra, a complex structure that lies in contact with the anterior wall of the air bladder. Between the angle formed by the two ribs and the centra of the second, third, and fourth vertebra, there remains an opening, the Weberian fenestra, through which the *tripus* passes. The ventral ribs of the fourth vertebra give off at their bases the *ossa suspensoria*, which together form a transverse plate (plate II, figure 2). The *tunica externa* of the air bladder is continuous with this plate.

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The third vertebra lacks transverse and ventral processes. A deep, vertical groove in the lateral surface of the centrum receives the articular process of the tripus.

The fourth vertebra possesses a single, much enlarged rib that passes ventrally and fuses, by its distal end, with the ventral rib of the second vertebra, to produce a structure that lies in contact with the anterior wall of the air bladder. Between the angle formed by the two ribs and the centra of the second, third, and fourth vertebrae, there remains an opening, the Weberian foramen, through which the aorta passes. The ventral ribs of the fourth vertebra give off at their bases the pars sustentaculi, which together form a transverse plate (plate II, figure 3). The tunica externa of the air bladder is continuous with this plate.

Chapter II

HISTORICAL SURVEY

Shortly after the initial description by Weber (1820) in his "De Aure et Auditu Hominus et Animalium. Pars. 1. De Aure Animalium aquatilium," the apparatus was described in various species by several authors. Among the early investigators, Huschke (1822), St. Hilaire (publication of 1824, cited from Watson, 1939), Müller (1842), and Beaudelot (1868) were notable.

From his work on Siluris glanis, Weber (1820) judged the function of the apparatus to be related to audition and concluded that the ossicles were homologous with the mammalian ear bones. He therefore referred to them as the auditory ossicles. Three of the individual bones he called the malleus, incus, and stapes after their supposed mammalian homologues. The fourth bone, with no supposed counterpart in the mammals, Weber termed the claustrum.

Many of the investigators of the nineteenth century shared Weber's view and adopted his terminology, but some did not. St. Hilaire (vide Watson, 1939, p. 464), Müller (1842, pp. 323-328) and Beaudelot (1868, pp. 87-109), for example, disputed the homology of the ossicles with the ear bones of mammals and instead considered them to be parts of the anterior vertebrae. However, it was Bridge and Haddon (1889, 1892) who gave impetus to this concept which later

replaced that of Weber. They described the Weberian apparatus of ninety-two species of siluroids and replaced the misnomer "auditory ossicles" with a new term, Weberian ossicles, in honor of the investigator who first described them. They proposed the now commonly used terms, tripus, intercalarium, and scaphium, as substitutes for the names properly associated with the mammalian ear bones. The term claustrum used by Weber for the additional bone was retained by these authors. Bridge and Haddon (1892, p. 143) also suggested a hydrostatic function for the air bladder and the ossicles.

Not only did Müller realize that the ossicles were derivatives of the vertebrae, but he also was the first to conclude that the Weberian organ had systematic importance. He concluded that several externally diverse groups, the siluroids, cyprinids, and characids, were related because of the presence of this complex apparatus. In 1885, Sagemehl (pp. 1-119) used the Weberian apparatus as a taxonomic character with which to separate many fish from their earlier categories and combine them in a new group, the Ostariophysi.

Certain investigators, especially Wright (1884, pp. 249-250) who worked with Amiurus [= Ictalurus] catus, believed that the Weberian ossicles were not entirely derived from the vertebrae, but that connective tissue ossifications also contributed to the definitive structures. Wright first discovered the fused condition of the second, third, and fourth vertebrae, a condition found to a greater or lesser extent in all the Ostariophysi.

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The nomenclature in common usage in the literature published in German is the result of the work of Thilo (1908). For the term malleus as used by Weber, Thilo (1908, pp. 780-787) proposed the term Hebel; for the incus, Lenker; for the stapes, Deckel; and for the claustrum, Einlage. Thilo's experiments with pressure convinced him that the function of the Weberian apparatus was hydrostatic.

The apparatus in a catostomid, *Ictiobus urus*, was described briefly by Adams (1928). Adams (1928, p. 117) concluded that only the first three vertebrae were involved in the Weberian apparatus and that it was the third rather than the fourth ventral rib that was highly modified around the tripus and the anterior end of the air bladder.

Chranilov (1927, 1930) used the Weberian apparatus as an aid in classification within the order Ostariophysi.

Two more recent works, dealing with morphology of the Weberian apparatus, treat with the Catostomidae. Krumholz (1943) studied the Weberian apparatus of North American ostariophysines including eleven members of the Catostomidae. Nelson (1948) compared the Weberian structures in catostomids with reference to their significance in systematics.

The first complete embryological study was that by Nusbaum in 1908 on *Cyprinus carpio*, family Cyprinidae. He thought that the first three vertebrae formed part of the skull and that the ossicles were entirely derived from the fourth and fifth vertebrae. According to Nusbaum's interpretation the intercalarium was derived from the fourth

vertebra and the tripus from the fifth.

Matveiev (1929, pp. 464-465) agreed with Wright that the ossicles originated both from modified vertebrae and from other connective tissue elements. Matveiev discovered in Scardinius erythropthalmus, a cyprinid, evidence that led him to conclude that both the intercalarium (1929, p. 501) and the scaphium (1929, p. 503) have dual origins.

The most recent work of an embryological nature is that of J. M. Watson (1939) on another cyprinid, the goldfish. In his excellent paper, Watson compared the development of the ossicles as seen in the goldfish with that described by previous authors. He corroborated the concept that both vertebral and connective tissue elements have a part in the origin of the ossicles. He (Watson, 1939, p. 453) discovered that an arch of cartilage dorsal to the first vertebra is continuous with the exoccipital bones of the cranium; this cartilaginous arch he called the "cartilage ring." In addition Watson (1939, p. 456) believed that the transverse processes of the second vertebra are homologous with dorsal ribs, that the ventral processes of the second and fourth vertebrae are homologous with pleural or ventral ribs, and that the ossa suspensoria are homologous with hemopophyses.

The Weberian mechanism has received considerable study of a morphological nature but much less attention has been given to developmental and physiological aspects. Among the studies concerned primarily with the function of the ossicles and the air bladder, the papers of Kuiper (1915)

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The Weberian apparatus has received considerable study of a morphological nature but much less attention has been given to developmental and physiological aspects. Among the studies concerned primarily with the function of the vesicles and the air bladder, the papers of [illegible] (1919)

and Evans (1925) are outstanding. Kuiper (1915, pp. 572-582) observed the reactions of fish to pressure changes, variations in light intensity, hydrostatic disturbances, and vibrations, both before and after the Weberian organ had been disrupted. He agreed with Weber that the organ was associated with the hearing of fishes. Evans (1925, pp. 547-574), studying many ostariophysines, discovered more evidence corroborating Weber's theory. Bridge and Haddon (1892, p. 142) thought the ossicular arrangement too lax to conduct rapid vibrations, but Evans found the Weberian organ capable of such. He also determined that it was possible for the air bladder to receive faint changes in the pressure impinging upon the sides of the fish. These changes were, he postulated, transferred to the internal ear by the ossicles. Subsequent investigators have been in agreement with Kuiper and Evans.

Other than the paper by Nelson (1948), which dealt with adult morphology and systematics, the Weberian apparatus of the family Catostomidae has had relatively little attention. The development of the apparatus in the catostomids apparently has not been studied previously. It is the purpose of this paper to describe the origins of the Weberian ossicles as seen in a common New Mexican catostomid, Pantosteus plebius, and to trace the developmental path of the Weberian apparatus to its mature condition.

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Chapter III

MATERIALS AND METHODS

The specimens used in this study were from two sources. The majority were collected during June, 1958, from the backwaters of the Jemez River and the San Antonio Creek near Jemez Springs, New Mexico. The remainder of the fish studied were reared from eggs obtained from adults taken in the two above mentioned streams. After artificial fecundation, the eggs were incubated and the young raised in tanks at 16° C. Eight days were required for development before hatching. The larvae at hatching were approximately 8 mm. long and three days later were 9 mm., the size at which cartilage formation was apparent in the vertebral column of field-collected specimens. However, in aquarium-raised specimens, cartilage did not appear in the vertebrae until the fish were 10 mm. long. In general the aquarium-raised fish were about 1 mm. longer than the stream-reared specimens in a comparable stage of development. Therefore, a fish raised indoors reached a length of 11 mm. before it showed the same bone formation as a fish of 10 mm. collected from the normal habitat. All measurements quoted in this paper were taken from field-collected specimens.

The larval fish were either fixed in Bouin's fluid and preserved in 70% alcohol or were fixed and stored in 10% formalin. Transverse, frontal, and sagittal sections for

microscopic examination were prepared by standard histological techniques. Methylene blue and alizarin stains yielded the best tissue differentiation.

To study the ossicular apparatus in adults and juveniles, several types of preparations were used. Skeletons of large adult fish were prepared by the use of dermestid beetles and were bleached in ammonia. Some skeletons were prepared by dipping specimens into boiling water and removing the softened flesh from the bones. Other adult specimens were treated with alizarin as a stain for bone, cleared with potassium hydroxide, and stored in glycerine. A fourth method, which differentiated between bone and cartilage, involved staining the cartilage with methylene blue, macerating the flesh with potassium hydroxide, and staining the bone with alizarin.

Development was traced from the condition found in the adult, through successively younger stages, to the earliest recognizable beginnings of the Weberian apparatus.

Chapter IV

DEVELOPMENT OF THE APPARATUS

The traditional belief concerning the development of the Weberian ossicles is that they represent modified portions of the first three vertebrae. Recent studies indicate a more complex history of development. In addition to modifications of the three anterior vertebrae, other connective tissue elements contribute to the rudiments of the ossicles. These non-vertebral elements are of two types, masses of mesenchyme cells in which membranous bone is later formed and ligament which later ossifies.

In an embryological study, the vertebral rudiments are best discussed in terms of the arcualia of the vertebrae rather than as ribs or transverse processes, because complete structures such as ribs are not present in the younger stages. The arcualia or arch components that fuse to form a typical vertebra are the basidorsal and interdorsal, which are paired components of the upper or neural arch, and the basiventral and interventral, which are paired components of the lower or hemal arch. Only the basidorsals and basiventrals are involved in ossicle construction.

Basidorsals and basiventrals may be either mesenchymatous or cartilaginous (Watson, 1939, p. 462), but both are entirely cartilaginous in Pantosteus plebius. The basidorsals and basiventrals develop first at the anterior end of the

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Basidorsals and basiventrals may be either precartilaginous or cartilaginous ([illegible], [illegible], p. [illegible]), but both are entirely cartilaginous in <u>Ictalurus [illegible]</u>. The basidorsals and basiventrals develop first at the anterior end of the

vertebral column and develop posteriorly in regular sequence. Basiventrals do not occur in the first and second vertebrae. The first basiventral to appear, the third, is a contributor to the *tripus*. It was found that ossification of the basidorsals and basiventrals does not occur until the fish reach a length of 30 mm. In *Pantosteus* there is no evidence of distinct interdorsals or interventrals.

In the discussion of the homologies of the various parts of the apparatus, it is necessary to refer to the time of development. Thus far, length in millimeters of fish collected from their normal habitat has been used to designate any given stage of development. Because there is difference in length of specimens at a definite level of ossicle formation between fish raised in aquaria and those raised in streams, the specimens have been designated according to five standard stages:

1. First appearance of the arcualia of the vertebrae as groups of cartilage cells lying alongside the notochord. This stage is approximately the same as Balinsky's "stage 32" (1948, p. 337).
2. Arcualia and connective tissue elements present in position of future ossicles.
3. Fusion of the elements of each ossicle complete.
4. Ossicles assume mature shape. Stages 2, 3, and 4 occur during and before Balinsky's "stage 33" (*Ibid.*).
5. Ossification of all ossicles complete. Balinsky's "stage 36" (*Ibid.*) is approximately the same as this stage.

The stages described by Balinsky, even though intended to apply to the Cyprinidae, fit the steps in development of

vertebral column and develop posteriorly in regular sequence. Basiventrals do not occur in the first and second vertebrae. The first basiventral to appear, the third, is a contributor to the tripus. It was found that ossification of the basidorsals and basiventrals does not occur until the fish reach a length of 50 mm. In *Pantosteus* there is no evidence of distinct interdorsals or interventrals.

In the discussion of the homologies of the various parts of the apparatus, it is necessary to refer to the time of development. Thus far, length in millimeters of fish collected from their normal habitat has been used to designate any given stage of development. Because there is difference in length of specimens at a definite level of Weberian formation between fish raised in aquaria and those raised in streams, the specimens have been designated according to five standard stages:

1. First appearance of the cartilages of the vertebrae as groups of cartilage cells lying alongside the notochord. This stage is approximately the same as Bolinsky's "stage 2a" (1946, p. 107).

2. Arcualia and connective tissue elements present in positions of future ossicles.

3. Fusion of the elements of each ossicle complete.

4. Ossicles assume mature shape. Stages 1, 2, 3, and 4 occur during and before Bolinsky's "stage 3a" (*Ibid.*).

5. Ossification of all ossicles complete. Bolinsky's "stage 3b" (*Ibid.*) is approximately the end of this stage.

The stages described by Bolinsky, were already intended to apply to the Cyprinidae, [illegible] development of

Pantosteus plebius fairly accurately (Koster, unpublished data).

The way in which the centrum of the vertebra develops is not clearly understood. The best explanation is that a cylinder of bone develops within a layer of mesenchyme that surrounds the notochord and its sheaths (Goodrich, 1930, pp. 43-44). The arcualia have only a small share in the formation of the centrum, but a thin layer of cartilage surrounds the bony layer at the 25 mm. stage.

Vertebral elements are the chief or sole contributors to three of the four ossicles: the *scaphium*, *intercalarium*, and *tripus*. The fourth bone, the *claustrum*, is derived entirely from mesenchyme and does not pass through a cartilaginous stage.

TRIPUS. The *tripus* (*malleus* of Weber), which is the most complex of the ossicles in its beginning, has a threefold origin. The major portion of the bone is contributed by the basiventral of the third vertebra. The articular process, body, and much of the anterior and posterior processes form from this beginning (plate VI, figure 2). The distal portion of the anterior process is formed by ossification within the interossicular ligament. That ossification of the posterior end of the interossicular ligament contributes to the anterior process can be detected by comparison of the length of the ligament in plates V, figure 1 and plate IX, figures 1 and 2. In early stages the transformator process can be seen to have an origin

[illegible] [illegible] fairly accurately (Gbdter, unpublished data).

The way in which the centrum of the vertebra develops is not clearly understood. The usual explanation is that a cylinder of bone develops within a layer of mesenchyme that surrounds the notochord and its sheath (Goodrich, 1930, pp. 43-46). The specimens have only [illegible] in the formation of the centrum, but a thin layer of cartilage surrounds the body layer at the earliest stage.

Vertebral elements are the origin of some contributions to three of the four ossicles: the scaphium, intercalarium, and tripus. The fourth bone, the claustrum, is derived directly from mesenchyme and does not pass through a cartilaginous stage.

TRIPUS. The tripus (malleus of Weber), which is the most complex of the ossicles in its beginning, has a threefold origin. The major portion of the bone is contributed by the basiventral of the third vertebra. The articular process, body, and much of the anterior and posterior processes form from this beginning (plate VI, figure 2). The distal portion of the anterior process is formed by ossification within the interossicular ligament. That ossification of the posterior end of the interossicular ligament contributes to the anterior process can be detected by comparison of the lengths of the ligament in plate V, figure 1, and plate IV, figures 4 and 5. In early stages the transformator process [illegible]

distinct from that of the rest of the *tripus*; this origin is shared with the more caudal part of the posterior ramus. The transformator rudiment, a mass of cartilage in the position of the dorsal rib of the third vertebra, is seen on plate VII, figure 2 before its fusion with the third basiventral.

The first rudiment of the *tripus* to appear is the third basiventral, which appears first at the 9 mm. stage. The additions to the anterior and posterior processes and the transformator rudiment are visible at about 9.5 mm. By the 10 mm. stage the various elements of the *tripus* have fused into a single, arch-shaped structure. Ossification of the bone is nearly completed by the 15 mm. stage, but the articular process remains cartilaginous through the 30 mm. stage.

INTERCALARIUM. The slender spicule of bone, called by Weber (1820, p. 87) the *incus* and by Bridge and Haddon (1889, p. 317) the *intercalarium*, is chiefly a derivative of the second vertebra. As in other Ostariophysi, the shaft of the *intercalarium* of *Pantosteus* arises from the basidorsal of the second vertebra. However, in agreement with Watson's (1939, p. 457) finding in the goldfish, the *intercalarium* of *Pantosteus* has a second origin. This is a small formation of bone, which forms the *manubrium incudis*, in the interosseous ligament. Thus, the *manubrium incudis* is membranous while the shaft is vertebral in origin. The two elements are distinct at 9.5 mm. but at 10 mm. are completely

distinct from that of the rest of the [illegible]. This region is shared with the more caudal part of the posterior [illegible]. The transformation [illegible], [illegible] of ossification in the portion of the [illegible] side of the [illegible] structure, [illegible] on plate VII, figure 2 before its fusion with the [illegible] basiventral.

The first rudiment of the [illegible] to appear is the [illegible] basiventral, which appears first at the 7 mm. stage. The additions to the anterior and posterior [illegible] the transformation [illegible] within it [illegible] mm. By the 10 mm. stage the various elements of the [illegible] have fused into a single, arch-shaped structure. Ossification of the bone is nearly completed by the 25 mm. stage, but the articular process remains cartilaginous through the [illegible] mm. stage.

INTERCALARIUM. The element [illegible], called by Weber (1820, p. [illegible]) the [illegible] and by [illegible] and [illegible] (1939, p. 317) the intercalarium, is actually a [illegible] of the second vertebra. At an [illegible], the anlage of the intercalarium of [illegible] arises from the [illegible] of the second vertebra. However, in agreement with [illegible]'s (1930, p. 457) finding in the [illegible], the intercalarium of [illegible] has a [illegible] origin. This is a small formation of bone, which forms the [illegible] ligament, in the intervertebral ligament. Thus, the [illegible] [illegible] is [illegible] while the [illegible] is vertebral in origin. [illegible] [illegible]

[illegible]

fused. The shaft of the bone has ossified by the 11 mm. stage. This is much earlier than ossification of the part of the basidorsal that forms the neural arch.

SCAPHIUM. Contrary to the results of other authors working upon different groups, the scaphium of Pantosteus has but a single contributing element, the basidorsal of the first vertebra. At the earliest stage at which a rudiment of the scaphium can be detected, there is a rod of cartilage, which consists of only a few cells, that projects anteriorly from the basidorsal. As seen in sagittal sections, the cartilaginous rod and the basidorsal proper make an L-shaped structure of cartilage. This cartilaginous rod is directed obliquely, dorso-anteriorly, from the horizontal plane, and as it falls in the same plane as do the walls of the concha stapedis in older specimens, we may conclude that the cartilaginous rod is the beginning of the concha stapedis. The rod is present within a mass of mesenchyme more densely arranged than the surrounding tissue. As development progresses, the basidorsal extends dorsally to form the second neural arch, and the cartilaginous rod, as it elongates beyond the concha stapedis, forms the anterior process of the scaphium.

The history of the scaphium begins in the 9 mm. stage when a few cartilage cells are seen lying next to the notochord. The definitive aspect of the bone is seen by 11 mm. and ossification is complete by the 15 mm. stage.

CLAUSTRUM. The claustrum, the smallest of the ossicles, also has a single origin. Historically, the origin of the

fused. The shaft of the bone was ossified by the 11 mm. stage. This is much earlier than ossification of the part of the basidorsal that forms the neural arch.

SCAPHIUM. Contrary to the results of other workers working upon different groups, the scaphium of [illegible] has but a single contributing element, the basidorsal of the first vertebra. At the earliest stage at which evidence of the scaphium can be detected, there is a rod of cartilage which consists of only a few cells, that originates [illegible] from the basidorsal. As seen in sagittal sections, the cartilaginous rod and the basidorsal present a continuous structure of cartilage. This cartilaginous rod is directed obliquely, dorso-anteriorly, from the basidorsal, and as it falls in the same plane as the walls of the concha stapedis in older specimens, we may consider that the cartilaginous rod is the beginning of the concha stapedis. The rod is present within a mass of mesenchyme more densely arranged than the surrounding tissue. As development proceeds, the basidorsal extends dorsally to form the second neural arch, and the cartilaginous rod, as it continues to form the concha stapedis, forms the anterior process of the scaphium.

The history of the scaphium begins in the [illegible] stage when a few cartilage cells are seen lying next to the notochord. The definitive aspect of the bone is reached by 11 mm. and ossification is complete by the [illegible] stage.

CLAUSTRUM. The claustrum, the smallest of the ossicles, also has a single origin. [illegible]

claustrum has been the biggest puzzle to investigators. It has been described as a derivative of the skull, as a part of the first neural arch, and as an accessory cartilage. In Pantosteus the claustrum has no formation in cartilage; it forms as a direct ossification of connective tissue elements, not connected with, nor derived from, the vertebrae. The anterior vertebrae are surrounded by a sheath, the saccus paravertebralis, that separates them from the musculature. The spinal cord is surrounded, in the position to be occupied by the sheath in later stages, by a thick layer of loosely packed mesenchyme. Clumping together of some of the cells of this layer dorsal to the scaphium, plus subsequent ossification, forms the claustrum. The rudiment of the claustrum lies dorso-lateral to the spinal cord and the adult bones occupy part of the position of the first neural arch.

The claustrum develops later than the other ossicles. The rudiment cannot be detected until 10 mm. (plate V, figure 2). Plate VII, figure 1 shows the ossicle partly mesenchymatous and partly osseous while the scaphium directly below is partly osseous and partly cartilaginous. A fully developed claustrum is not present until the 12 mm. stage.

INTEROSSICULAR LIGAMENT. During the time when the scaphium, intercalarium, and tripus are taking form, the interossicular ligament, which connects the three, makes its appearance. Dense mesenchyme is present in the future position of the ligament by 9.5 mm. and the fibrous nature

claustrum has been the biggest [illegible] has been described as [illegible] of the first neural arch, and [illegible]. In the Pantosteus the claustrum [illegible] forms as a direct [illegible] not connected with [illegible] anterior vertebrae [illegible], the parvertebrals, that [illegible]. The spinal cord is surrounded, [illegible] by the sheath in later stages, [illegible] packed mesenchyme. [illegible] this layer dorsal to the [illegible] forms the claustrum. The rudiment of the claustrum [illegible] dorso-lateral to the spinal cord and [illegible] part of the portion of the first neural arch.

The claustrum develops later than [illegible]. The rudiment cannot be detected [illegible] figure 2). Plate VI, [illegible] mesenchymatous [illegible] directly below [illegible]. A fully developed claustrum is not [illegible] stage.

INTEROSSICULAR LIGAMENTS. [illegible] scaphium, intercalarium, and tripus [illegible] interossicular ligaments, which [illegible] its appearance. [illegible] position in the [illegible]

of the ligament is clear in sections of 10 mm. fish (plate V, figure 1).

PARS SUSTENTACULUM. The only representative of the vertebral column in Pantosteus of 8 mm. and younger stages is the notochord. The arcualia first appear around the notochord at 9 mm.

The first vertebra develops neither ribs nor neural arch. The spinal canal in this region is roofed over by an arch, the so-called "ring of cartilage." The "cartilage ring" is not visible in sections until the ossicles are completely formed. In fish of 12 mm. the "cartilage ring" is visible above the spinal cord as an extension of the cranium. By 20 mm. the "cartilage ring" extends posteriorly to the junction of the first and second centra. The more lateral parts of the area normally occupied by the first neural arch are formed by the claustra, which are mostly posterior to the "cartilage ring" in sections of 10 mm. to 12 mm. Pantosteus. The component, which in the trunk vertebrae would ordinarily form the neural arch, gives rise to the scaphium.

The second vertebra develops the two ribs typical of a fish vertebra, a dorsal or intermuscular rib, and a ventral or pleural rib. The dorsal rib develops in the junction of the horizontal and vertical skeletogeneous septa. The pleural rib is directed posteriorly as well as ventrally. During development the two pleural ribs of the second vertebra broaden, flatten, and fuse to form a plate, inclined

45^{o} from the long axis of the body (plate I, figure 3). It is at the ventral edge of this plate that the pleural ribs of the fourth vertebra join. The esophageal processes arise medially to the suture line of the second and fourth ribs and project posteriorly and slightly ventrally. Part of the basidorsal, or neural arch, element of this vertebra is modified to form the <u>intercalarium</u> and a part forms the anterior section of the cartilaginous mass that develops into the compound neural arch and spine of the second, third, and fourth vertebrae. In young stages the centrum of the second vertebra is distinct from those of the third and fourth vertebrae, but later the three centra fuse into the so-called "compound vertebra" of the adult. However, even in the adult, the sutures are visible.

The third vertebra lacks processes of any type. The neural arch elements fuse with those from the second and fourth vertebrae to form a compound neural arch and spine. The ventral part of the arch flares laterally from the centrum and gives the appearance of a short transverse process. The ventral and dorsal ribs of this vertebra are absent. Their rudiments, the basiventral and a mass of cartilage in the position of the dorsal rib, form much of the <u>tripus</u>.

The fourth vertebra develops a single pair of processes, the ventral ribs, that develop into expanded plates and fuse with the ventral ribs of the second vertebra. This accessory part of the Weberian apparatus appears at the

45° from the long axis of the body [illegible] is at the ventral edge of this plate [illegible] of the fourth vertebra [illegible] arise medially to the [illegible] ribs and project posteriorly [illegible] of the basidorsal, or neural arch, [illegible] is modified to form the intercalarium [illegible] anterior [illegible] into the compound neural [illegible] and fourth vertebrae. [illegible] second vertebra [illegible] fourth vertebrae, but later [illegible] so-called "compound vertebra" [illegible] in the adult, the sutures are [illegible].

The third vertebra lacks [illegible] neural arch elements [illegible] fourth vertebrae [illegible]. The ventral part of [illegible] centrum and gives the appearance [illegible] process. The ventral [illegible] absent. Their rudiments, [illegible] cartilage in the [illegible] the tripus.

The fourth vertebra develops [illegible] the ventral ribs, [illegible] fuse with the ventral [illegible] accessory [illegible]

same time as the ossicles and by the 12 mm. stage is completely formed in cartilage. The rudiments of the <u>ossa suspensoria</u>, which are projections from the bases of the ventral or pleural ribs, can be seen next to the anterior wall of the air bladder at 10 mm.

Chapter V

DISCUSSION

Three general theories of the origin and development of the Weberian apparatus have been proposed. Weber (1820) believed the ossicles to be homologous with the mammalian ear bones; St. Hilaire (1824), Müller (1842), Beaudelot (1868), Bridge and Haddon (1893), and Nusbaum (1908) thought the ossicles were formed by simple separations of parts of the anterior vertebrae; and Wright (1884), Matveiev (1929), and Watson (1939) concluded that connective tissue ossifications supplemented the modified portions of the vertebrae in the development of the ossicles. The results of this study are in accord with the last theory. The dual origin is best seen in the _intercalarium_ (plate IV, figure 2).

The development of the ossicles in _Pantosteus_ is very rapid; the passage from stages one to four in any one series occurs within the length increase of a single millimeter. The basidorsals and basiventrals appear at 9 mm. and the two previously described elements that fuse to form the _intercalarium_ have joined by the 10 mm. stage. Most of the very rapid development of the ossicles takes place between the lengths of 9.5 mm. and 10 mm. Watson (1939, pp. 452, 458) found cartilaginous basidorsals and basiventrals in goldfish of 8 mm., but the elements of the _intercalarium_ were not fused until 15 mm.

CHAPTER V

DISCUSSION

Three general theories on the [illegible] development of the Weberian apparatus [illegible]. [illegible] believed the ossicles to be homologous with [illegible] ear bones; St. Hilaire (1824), [illegible], [illegible] (1868), Bridge and Haddon (1893), and [illegible] the ossicles were formed by [illegible] of parts of the anterior vertebrae; and [illegible] and Watson (1939) concluded that [illegible] ossifications supplemented the neural [illegible] of the vertebrae in the development of the ossicles. [illegible] of this study are in accord with the [illegible] origin is best seen in the *intercalarium* [illegible].

The development of the ossicles in *Carassius* [illegible] rapid; the passage from stages [illegible] occurs within [illegible] length increase of [illegible]. The basidorsals and [illegible] of [illegible] and two previously [illegible] *intercalarium* have [illegible] by the [illegible] stage. [illegible] very rapid development of the [illegible] the lengths of 9.5 [illegible] and [illegible] (1939, [illegible] 453) found cartilaginous [illegible] in the goldfish of [illegible] mm, but the elements of the *intercalarium* were not fused until [illegible] mm.

All three of the origins of the *tripus* have been disputed. Krumholz (1943, p. 36) concluded that the *tripus* articulated with the second vertebra, but plate VII, figure 2 shows that much of the bone is derived from the basiventral of the third vertebra, and therefore, it is the third vertebra with which the *tripus* articulates. The transformator process has been called an ossification in the *tunica externa* of the air bladder (Wright, 1884, p. 249) or this plus the dorsal rib of the third vertebra (Watson, 1939, p. 461). The transformator process arises at the junction of the third myoseptum with the horizontal skeletogenous septum, the normal position of a dorsal rib. Since the third vertebra of the adult bears no ribs, we may conclude that part of the transformator process represents the third dorsal rib. Plate VII, figure 2 shows the basiventral and rib rudiments before their fusion. Watson (1939, p. 461) believed the anterior process of the *tripus* to be lengthened by ossification in the interossicular ligament, a process that is also seen in *Pantosteus*.

The two elements that contribute to the *intercalarium* are entirely separate at stage two (plate IV, figure 2). Plate IV, figure 2 also demonstrates that ossicle formation involves both vertebral and other connective tissue elements. A connective tissue contributor (lateral element seen on plate IV, figure 2) is the *manubrium incudis*, and the vertebral element is the basidorsal (plate IV, figure 2) of the second vertebra. The next stage in the development of

All three [illegible] origins of the tripus have been [illegible] Krumholz (1942, p. 26) concluded that the tripus articulates with the second vertebra, but Plate VII, figure [illegible] shows that much of the bone is derived from the [illegible] rib of the third vertebra, and therefore, it is the third vertebra with which the tripus articulates. The transformator process has been called an ossification in the tunica externa of the air bladder (Wright, 1884, p. 247) or [illegible] of the third vertebra (Watson, 1939, p. [illegible]). The transformator process arises at the junction of the third myoseptum with the horizontal skeletogenous septum, the normal position of a dorsal rib. Since the third vertebra of the adult bears no ribs, we may conclude that part of the transformator process represents the third dorsal rib. Plate VII, figure 2 shows the [illegible] before their fusion. Watson (1939, p. [illegible]) [illegible] the anterior process of the tripus to be [illegible] by ossification in the intercostal ligament, [illegible] that is also seen in *Pantosteus*.

The two elements that contribute to the *intercalarium* are entirely separate at stage two (Plate IV, figure [illegible]). Plate IV, figure 2 also demonstrates that [illegible] involves both vertebral and [illegible] A connective tissue [illegible] plate IV, figure 2) is the [illegible]. The vertebral element is [illegible] (plate IV, figure [illegible]) the second vertebra. The [illegible]

the *intercalarium* is the fusion of the two parts, as shown on plate VIII, figure 1.

There are two parts to the *scaphium*, which seemingly develop independently of one another during the first stages, but it is questionable if there are two distinct origins for the bone. According to Matveiev (1929, p. 503) a mass of mesenchyme appears anterior to the basidorsal, and ossification in this mass produces the *concha stapedis* or cup-shaped part of the *scaphium*. Watson (1939, p. 457) concurs with this idea. Sections of *Pantosteus* show a mass of mesenchyme and within this a cartilaginous rod that is continuous with the cartilage of the basidorsal. If the cartilaginous rod were distinctly separate from the basidorsal, we might conclude a dual origin for the *scaphium*, but at its first appearance the rod is continuous with the basidorsal. Therefore, it seems more likely that the *scaphium* arises from a single rudiment, the first basidorsal.

The first vertebra of *Pantosteus* has no neural arch. Because the cartilaginous "ring" that lies dorsal to the spinal cord is continuous with the cranium, the origin in *Pantosteus* is in agreement with Watson's (1939, p. 453) explanation that the "ring" is an extension of the exoccipitals.

A second cartilaginous mass becomes visible earlier in development than does the "cartilage ring" and forms a roof over the spinal cord dorsal to the anterior half of the second vertebra. The fate of this mass is to become part of the larger mass of cartilage that forms the compound neural

the intercalarium to the fusion of the two parts, as shown on plate XLIX, figure 1.

There are two parts to the scaphium which probably develop independently of one another during the first stages, but it is questionable if there are two separate centers of the bone. According to Matveiev (1929, p. 172) a mass of mesenchyme appears anterior to the basidorsal, and the ossification in this mass produces the crus anterior, the cup-shaped part of the scaphium (plate XLIX, figure [illegible]). [illegible] concurs with this idea. Watson [illegible] of scaphium [illegible] a mass of mesenchyme and within this a cartilaginous [illegible] which is continuous with the cartilage of the basidorsal. If the cartilaginous and [illegible] of [illegible] arise from the basidorsal, we might consider [illegible] to the scaphium, but at its first appearance the [illegible] is continuous with the basidorsal. Therefore, it seems logical [illegible] the scaphium arises from a single [illegible] (plate [illegible], figure [illegible]) [illegible].

The first [illegible] the [illegible] Because the cartilaginous [illegible] spinal cord is [illegible] the [illegible] Pantosteus is in agreement with [illegible] (1929, p. 172) explanation that the [illegible] of the [illegible].

A second [illegible] development then uses the [illegible] over the spinal cord [illegible] of the second vertebra. The [illegible] the [illegible]

spine and arch of the second, third, and fourth vertebrae. The entire mass has been interpreted as a part of the skull (Nusbaum, 1908, pp. 530-531), as a complex of the neural spines of the first three vertebrae plus the first three interspinous bones (Matveiev, 1929, p. 477), and as a complex of the basidorsals of the second, third, and fourth vertebrae plus probable contributions from the first three interspinous bones (Watson, 1939, p. 455). Sections of Pantosteus show that there is no connection between the cartilaginous mass and the skull. Findings of this study are in agreement with Watson's results concerning contributions from the basidorsals, but there are no indications of distinct interspinous bones. Therefore, it is necessary to attribute the cartilaginous mass to the basidorsals.

The modified ventral process of the fourth vertebra has had a complex history of suggested homologues. The vertebra possessing this complex ventral process has been called the third vertebra by Adams (1928, p. 117) and Krumholz (1943, p. 36), probably because they did not recognize that the second and third centra are fused. The process has been termed a hemal process (Adams, 1928, p. 117), a transverse process (Wright, 1884, p. 250; Bridge and Haddon, 1889, p. 311), and a pleural or ventral rib (Watson, 1939, p. 456). Because the modified ventral process articulates with the centrum in the fashion typical of a pleural rib as described by Goodrich (1939, pp. 34-44), one may conclude that the process represents a pleural rib.

spine and arch of the atlas [illegible]. The entire mass has been interpreted as a part of [illegible] (Nusbaum, 1908, pp. [illegible]) [illegible] spines of the first three vertebrae plus the [illegible] interspinous bones [illegible] of the basidorsals of the second [illegible] plus probable [illegible] bones (Watson, 1939, p. [illegible]). [illegible] that there is no connection between the [illegible] and the skull. [illegible] with Watson's remarks concerning [illegible] of the basidorsals, [illegible] interspinous bones. [illegible] it is [illegible] the cartilaginous [illegible] to the [illegible].

The modified ventral process of the fourth vertebra has had a complex history of [illegible]. The [illegible] possessing [illegible] ventral [illegible] of the third vertebra by [illegible] and [illegible] p. 369, probably because they did not recognize that the second and third centra are fused. [illegible] termed a basal process ([illegible], p. [illegible]), a [illegible] process (Wright, 1884, p. [illegible] and Watson, [illegible], p. 311), and a [illegible]. Because the modified ventral process [illegible] of the centrum in the [illegible] by Goodrich (1930, pp. [illegible]), and [illegible] the process [illegible]

The ventral rib of the second vertebra, like that of the fourth, is clearly homologous with the pleural rib of the trunk vertebrae, both because of its type of articulation and because of its position internal to the hypaxial muscle mass.

The horizontal projection of the second vertebra has been termed a transverse process by most early investigators and a dorsal rib by Watson (1939, p. 456). In _Pantosteus_ the position of the projection at the junction of the myoseptum with the horizontal skeletogenous septum verifies its homology with a dorsal rib.

The _ossa suspensoria_ occupy the position of hemopophyses, projections from the ventral side of the centrum representing remnants of the hemal arch, and are so termed by Watson (1939, p. 456). However, even during development, the _os suspensorium_ is never distinct from the fourth pleural rib.

The esophageal processes are mentioned only by Matveiev (1929, p. 482) and Nelson (1948, p. 230) and are difficult to determine as to their homology. The process arises from the second rib component of the second and fourth pleural rib complex. As the plate formed by the two pleural ribs of the second vertebra is uninterrupted, it is possible that the esophageal processes represent extensions of the hemopophyses incorporated into the flat plate. It is also possible that in many ostariophysines the esophageal processes have no homologues but have evolved separately in those groups which possess a very complex Weberian apparatus.

Chapter VI

SUMMARY

The development of the Weberian ossicles, a series of small bones that carries vibrations from the air bladder to the internal ear, was studied in various stages of the Rio Grande mountain sucker, *Pantosteus plebius*, a member of the family Catostomidae.

The Weberian apparatus in the adult has two divisions, a *pars auditum*, which consists of the Weberian ossicles proper, and the *pars sustentaculum*, which consists of the modified four anterior vertebrae that support the ossicles.

Microscopic examination of sections through young fish indicated that the ossicles are derived from the arcualia or arch components of the first three vertebrae plus other connective tissue elements. The *tripus* has a threefold origin, from the basiventral of the third vertebra, from a mass of cartilage in the position of the dorsal rib of the third vertebra, and from an ossification of the posterior end of the interossicular ligament. The *intercalarium* forms from two beginnings, the basidorsal of the second vertebra and an ossification in the center of the interossicular ligament. The *scaphium* has a single origin, the first basidorsal. The *claustrum* is derived by direct ossification within a mass of mesenchyme that is apparently unrelated to any vertebra. Except that the *scaphium* has a single rather than a dual origin, the development of the ossicles in the

Chapter [illegible]

[illegible]

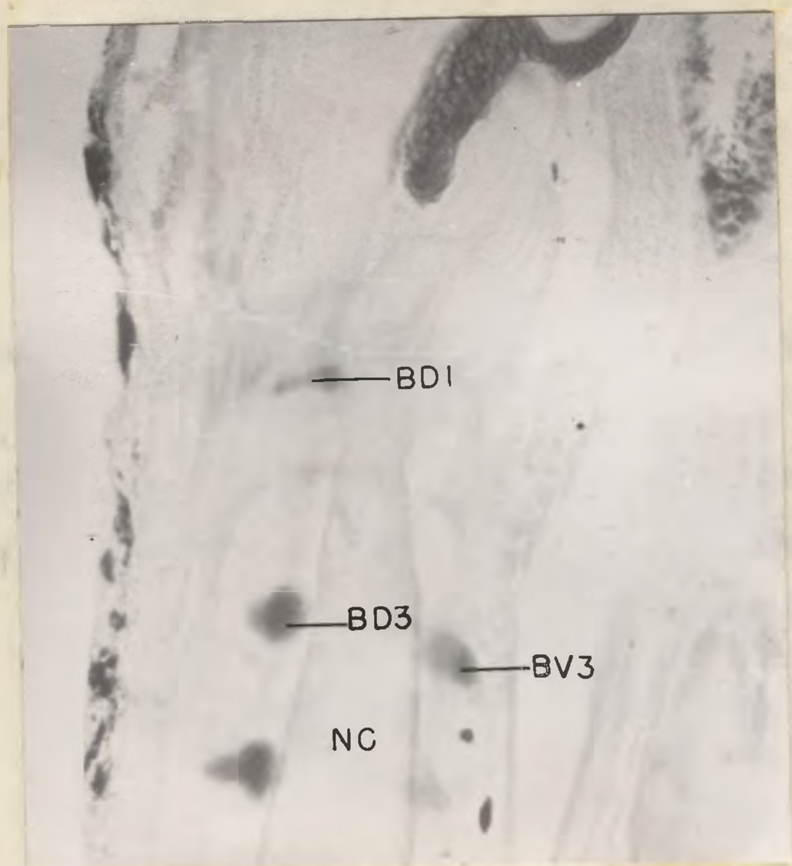
The development of the Weberian ossicles, a [illegible] small bones that carried vibrations [illegible] the [illegible] to the internal ear, was studied [illegible] of the [illegible] Grande mountain sucker, *Pantosteus [illegible]*, a [illegible] family Catostomidae.

The Weberian apparatus [illegible] a *pars auditum*, which [illegible] Weberian [illegible] proper, and the *pars [illegible]*, which [illegible] modified [illegible] the [illegible]

Microscopic examination [illegible] indicated that the [illegible] or arch components of the first [illegible] connective tissue [illegible] The [illegible] origin, [illegible] mass of cartilage in the [illegible] third vertebra, and [illegible] end of the [illegible] The [illegible] forms from two [illegible] vertebra and a [illegible] the [illegible] ligament. The [illegible] basidorsal. The [illegible] within a mass of [illegible] any vertebra [illegible] than a dual [illegible]

catostomid species Pantosteus plebius is similar to that described for the two cyprinids, Carassius (Watson, 1939) and Scardinius (Matveiev, 1929).

Development of the pars sustentaculum in Pantosteus does not vary greatly from that described by Watson (1939) in the goldfish, even though the anterior vertebrae are considerably more modified in the suckers. The cartilaginous "ring" over the first vertebra is a cranial extension and the mass of cartilage that forms the neural arch and spine of the second, third, and fourth vertebrae is definitely formed from the basidorsals of those vertebrae. The ventral processes of the second and fourth vertebrae fuse and form the Weberian fenestra and a complex rib combination. These ribs are true pleural ribs. The ossa suspensoria represent hemopophyses of the fourth vertebra. The homology of the esophageal processes is not clear, but they may represent hemopophyses of the second vertebra or may have evolved separately and without involving vertebral elements.


BD1
BD3
BV3
NC

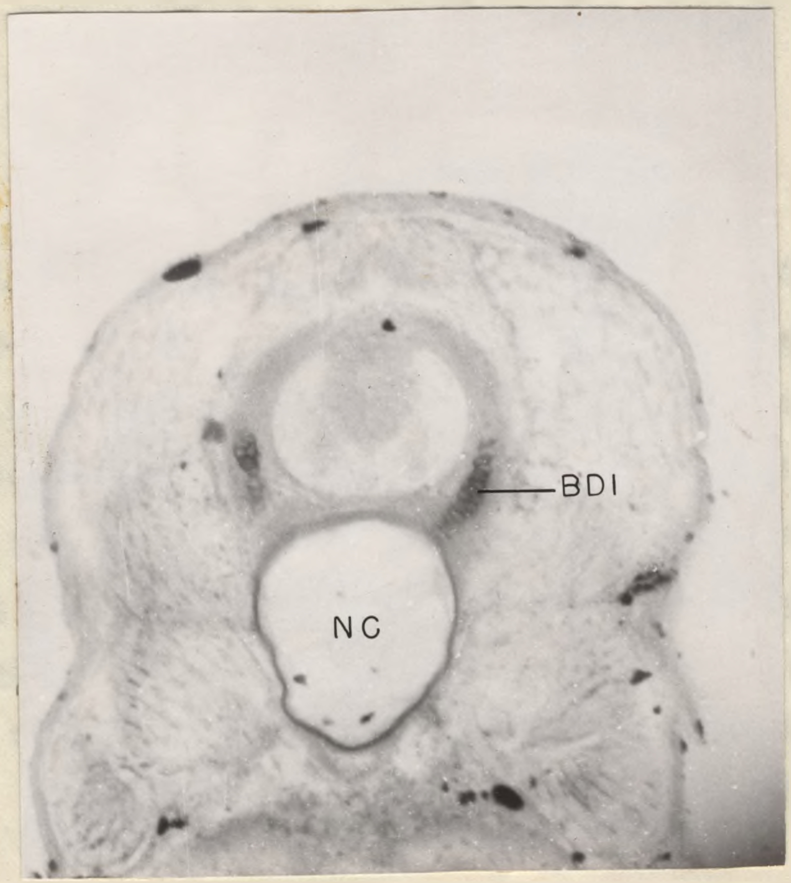
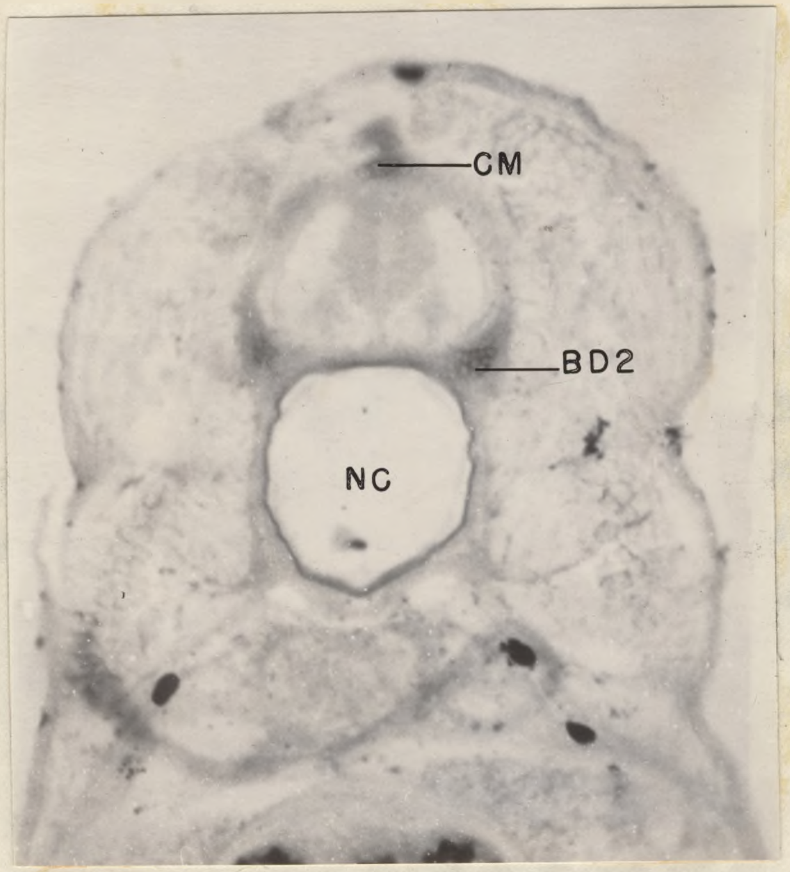

BD1
NC

Plate IV, Figure 1: Photomicrograph of a transverse section of a Pantosteus embryo at stage 1 (text, pp. 22-23); methylene blue and alizarin stains; orange filter; 150 X. BD2, basidorsal of the second vertebra; CM, cartilaginous mass; NC, notochord.

Plate IV, Figure 2: Photomicrograph of a frontal section of a Pantosteus embryo at stage 2 (text, pp. 22-23); methylene blue stain; orange filter; 150 X. BD1, basidorsal of the first vertebra; BD2, basidorsal of the second vertebra; BD3, basidorsal of the third vertebra; CS, concha stapedis portion of the scaphium; MI, manubrium incudis. The two elements that contribute to the intercalarium, the second basidorsal and the manubrium incudis, are visible before fusion.


CM
BD2
NC

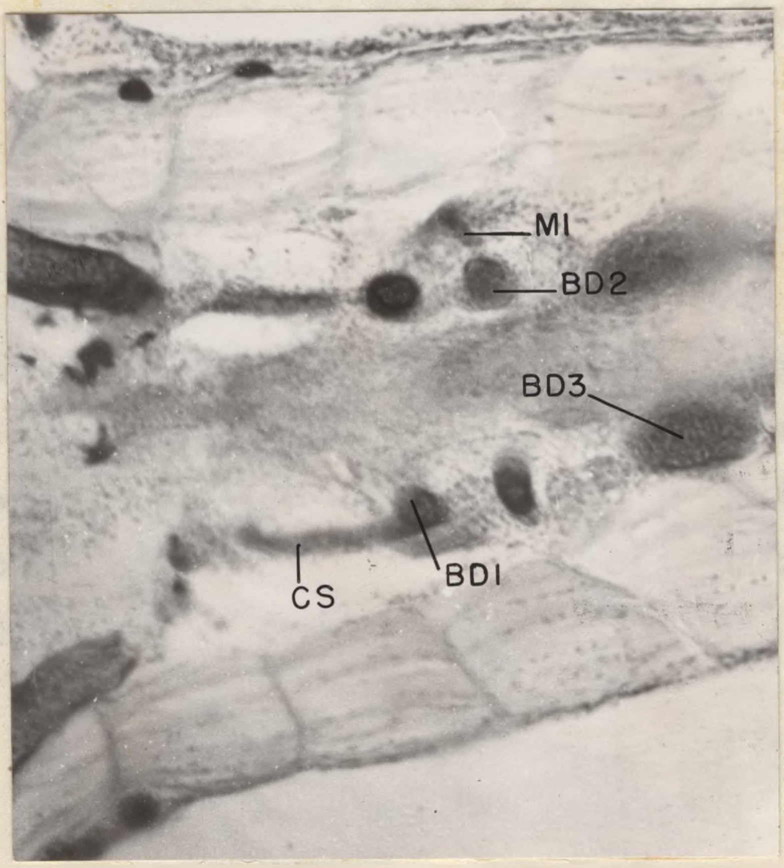

MI
BD2
BD3
CS
BD1

Plate V, Figure 1: Photomicrograph of a frontal section of a *Pantosteus* embryo at stage 3 (text, pp. 22-23); methylene blue stain; orange filter; 150 X. BD2, basidorsal of the second vertebra; IL, interossicular ligament; S, *scaphium*.

Plate V, Figure 2: Photomicrograph of a sagittal section of a *Pantosteus* embryo at stage 2 (text, pp. 22-23); methylene blue stain; orange filter; 150 X. BD1, basidorsal of the first vertebra; BD2, basidorsal of the second vertebra; BD3, basidorsal of the third vertebra; Cl R, rudiment of the *claustrum*; CM, cartilaginous mass.

Plate V, Figure 1: Photomicrograph of a frontal section of a Pantosteus embryo at stage 2 (text, pp. 22-23); methylene blue stain; orange filter; 150 X. BD2, basidorsal of the second vertebra; IL, interossicular ligament; S, scaphium.

Plate V, Figure 2: Photomicrograph of a sagittal section of a Pantosteus embryo at stage 2 (text, pp. 22-23); methylene blue stain; orange filter; 150 X. BD1, basidorsal of the first vertebra; BD2, basidorsal of the second vertebra; BD3, basidorsal of the third vertebra; Cl R, rudiment of the claustrum; CM, cartilaginous mass.


S
IL
BD2

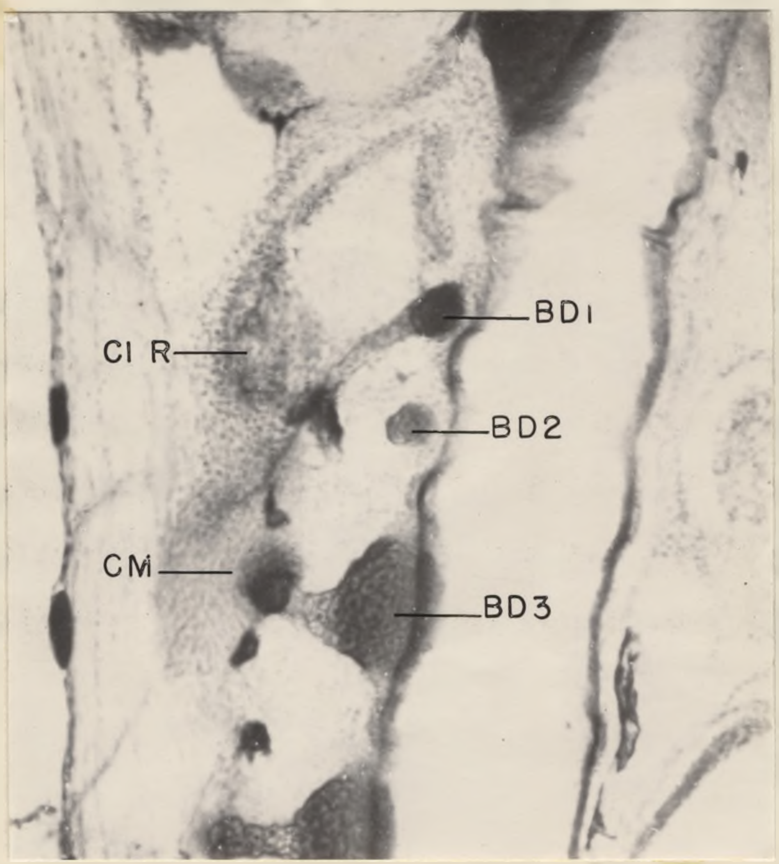

BD1
CI R
BD2
CM
BD3

Plate VI, Figure 1: Photomicrograph of a sagittal section of a *Pantosteus* embryo at stage 2 (text, pp. 22-23); methylene blue and alizarin stains; orange filter; 150 X. BD1, basidorsal of the first vertebra; Sk. skull. The rod of cartilage that extends anteriorly from the first basidorsal is visible on this photograph. This rod of cartilage is the rudiment of the *concha* *scaphium* portion of the *scaphium*.

Plate VI. Figure 2: Photomicrograph of a sagittal section of a *Pantosteus* embryo at stage 2 (text, pp. 22-23); methylene blue stain; orange filter; 150 X. BV3, basiventral of the third vertebra; BV4, basiventral of the fourth vertebra; Tr. T, rudiment of the transformator process of the *tripus*, which is the dorsal rib of the third vertebra.

Plate VI, Figure 1: Photomicrograph of a sagittal section of a Pantosteus embryo at stage 2 (text, pp. 22-23); methylene blue and alizarin stains; orange filter; 150 X. BD1, basidorsal of the first vertebra; Sk, skull. The rod of cartilage that extends anteriorly from the first basidorsal is visible on this photograph. This rod of cartilage is the rudiment of the concha stapedis portion of the scaphium.

Plate VI, Figure 2: Photomicrograph of a sagittal section of a Pantosteus embryo at stage 2 (text, pp. 22-23); methylene blue stain; orange filter; 150 X. BV3, basiventral of the third vertebra; BV4, basiventral of the fourth vertebra; Tr. T, rudiment of the transformator process of the tripus, which is the dorsal rib of the third vertebra.


SK
BDI

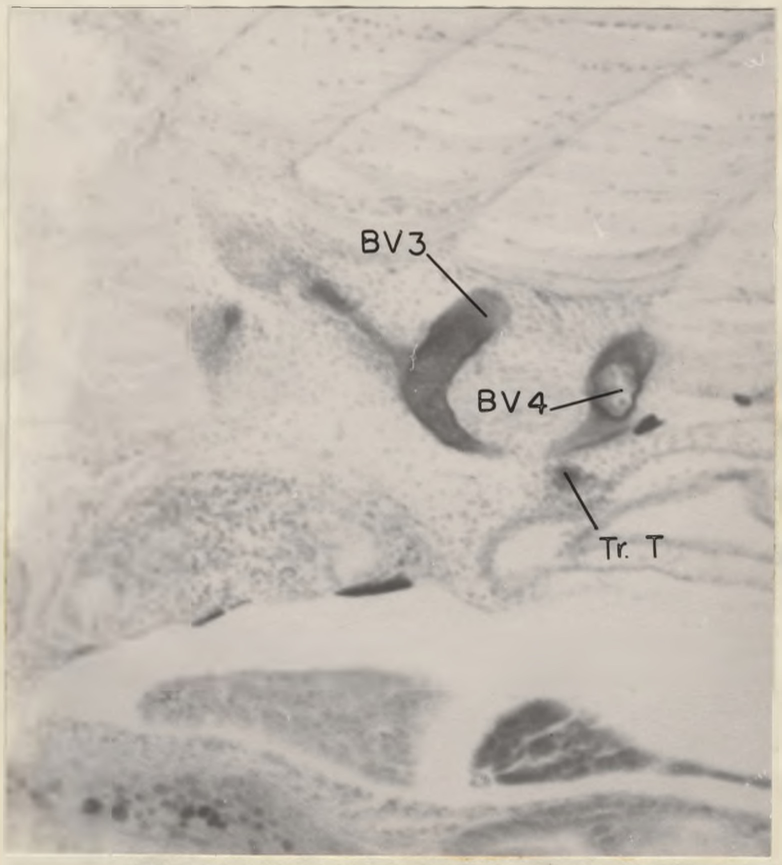
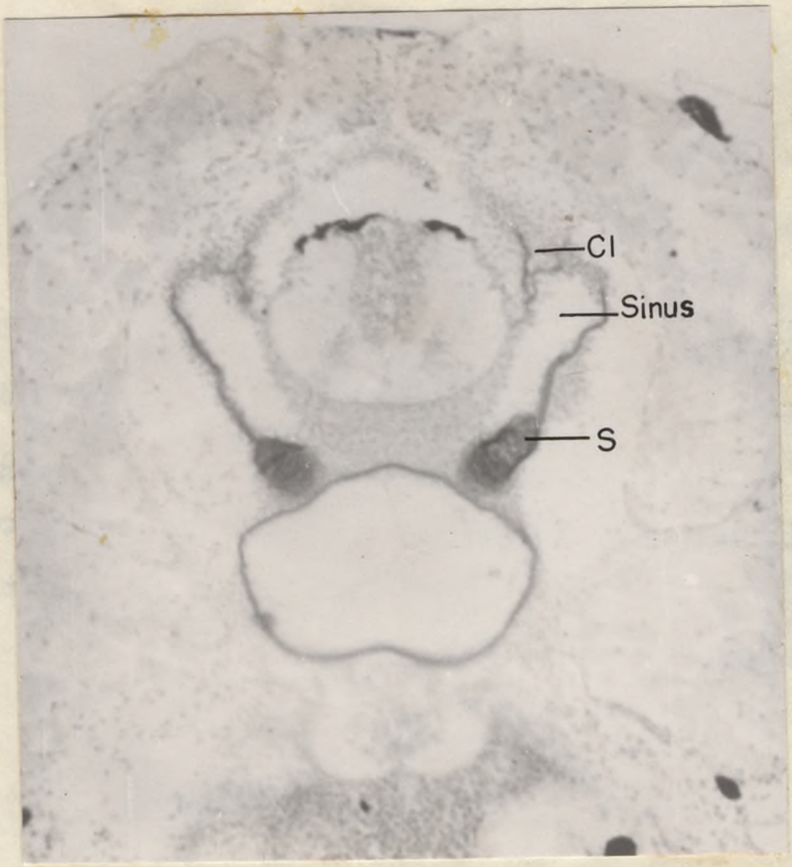

BV3
BV4
Tr. T

Plate V[illegible] al section
of a [illegible] 23);
methylen[illegible] , basiventral
of the [illegible] fourth
vertebra[illegible] process of
the [illegible] vertebra.


Cl
Sinus
S


CR
Ml

Plate VII [illegible] erse
section [illegible] pp. 22-23);
methylene [illegible] claustrum;
S, *scaphi*[illegible]

Plate VII [illegible] erse
section [illegible] pp. 22-23);
methylene [illegible] "cartilage
ring"; Ml [illegible]

Plate VIII, Figure 1: Photomicrograph of a sagittal section of a Pantosteus embryo at stage 3 (text, pp. 22-23); methylene blue stain; orange filter; 150 X. Cl, claustrum; I, intercalarium; S, scaphium. The parts that form the ossicles have fused and are shown in their definitive shapes on this photograph.

Plate VIII, Figure 2: Photomicrograph of a sagittal section of a Pantosteus embryo at stage 4 (text, pp. 22-23); methylene blue stain; orange filter; 150 X. Ant. T, anterior process of the tripus; IL, interossicular ligament; MI, manubrium incudis; S, scaphium.


Cl
S
I

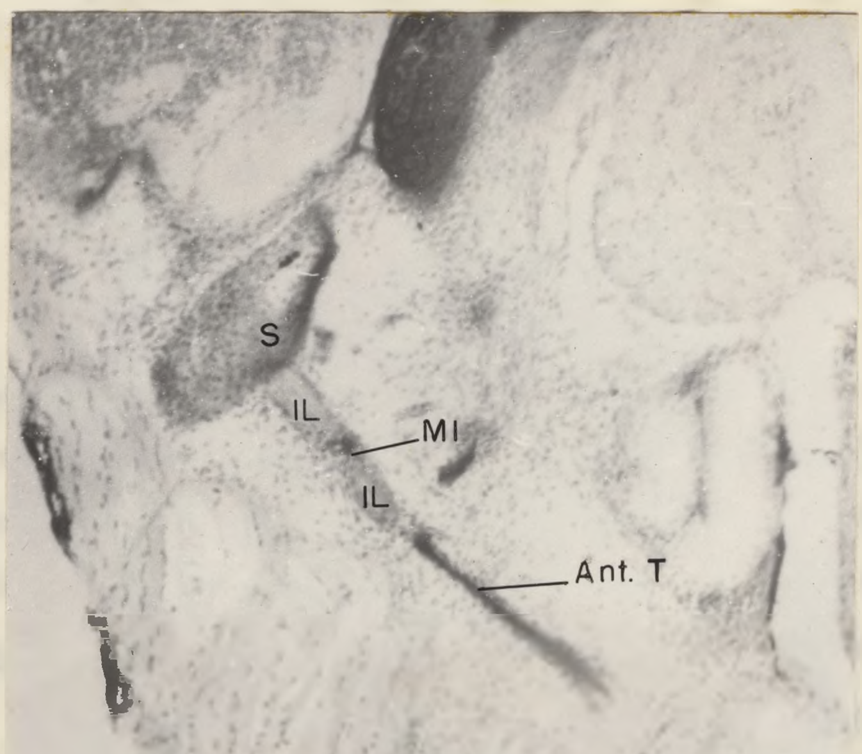

S
IL
MI
IL
Ant. T

Plate [illegible] al section
of a [illegible]);
methy [illegible] *ilanatrom*;
I. *In* [illegible] n the
nasin [illegible] ive
shape [illegible]

Plate [illegible] ial section
of a. [illegible] 3);
methy [illegible] T,
anter [illegible] ligament;
NI, [illegible]

Plate IX, Figure 1: Photomicrograph of a frontal section of a Pantosteus embryo at stage 5 (text, pp. 22-23); methylene blue and alizarin stains; 120 X. Cl, claustrum; S, scaphium; SC, spinal cord.

Plate IX, Figure 2: Photomicrograph of a frontal section of a Pantosteus embryo at stage 5 (text, pp. 22-23); methylene blue and alizarin stains; 120 X. BD1, basidorsal of the first vertebra; BD2, basidorsal of the second vertebra; BV3, basiventral of the third vertebra; I, intercalarium; S, scaphium.


CI
S
SC

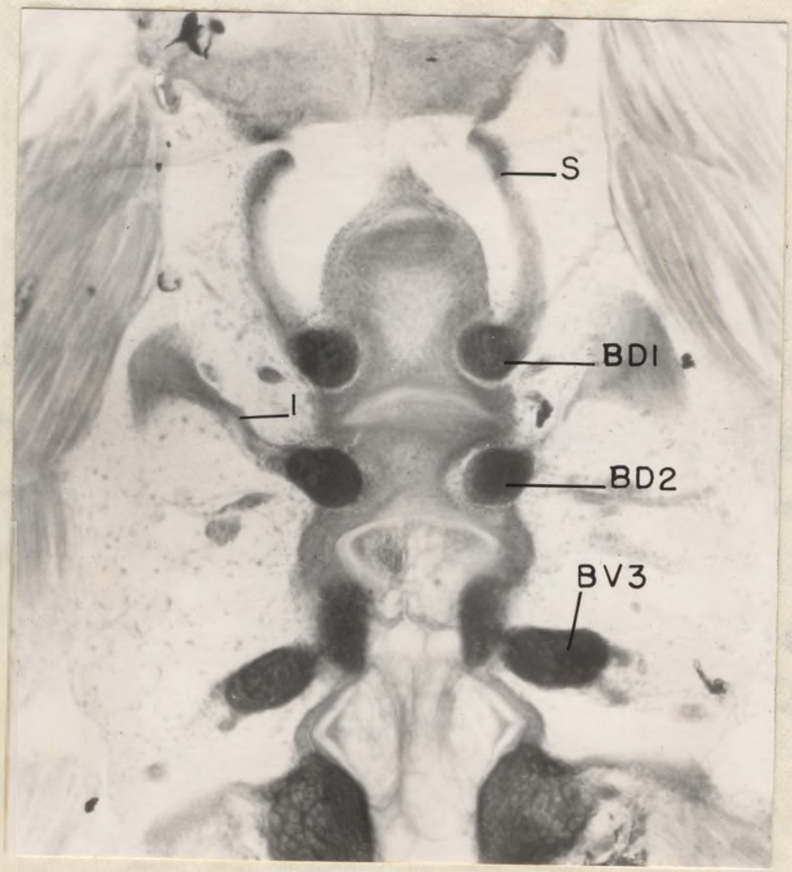
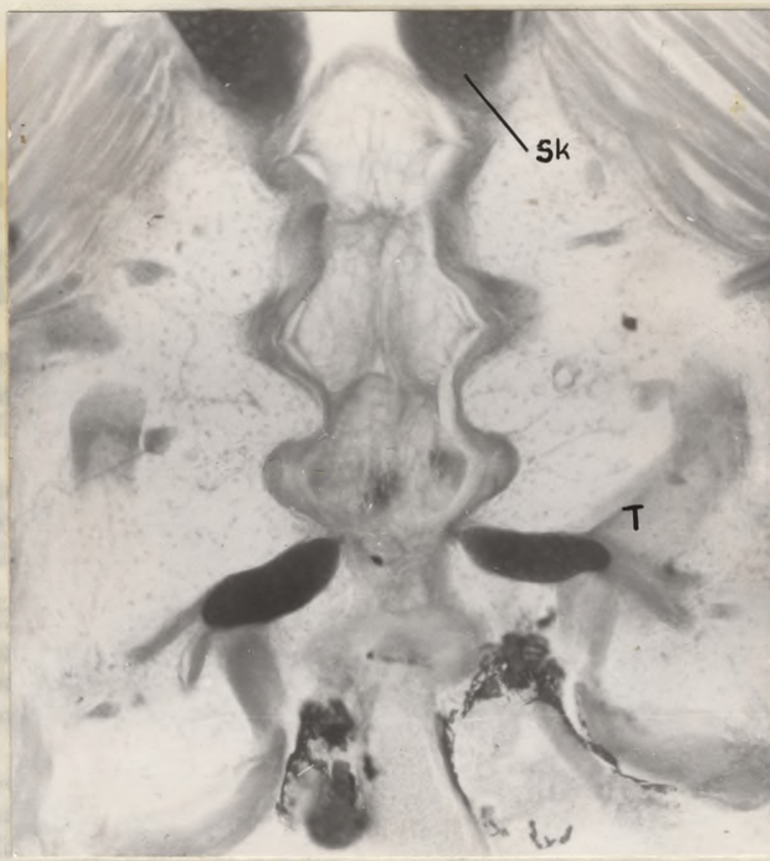

S
BD1
I
BD2
BV3

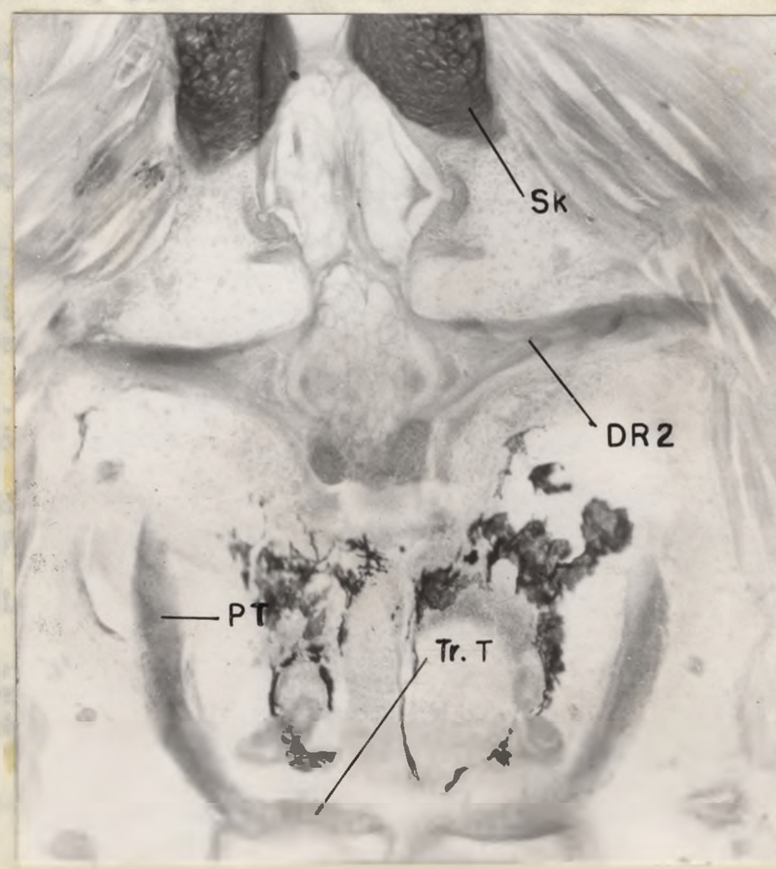
Plate 1? ... section
of a Ran... ...);
methylene ... ...
5, ...

Plate 1? ... section
of a ... ...);
methylene ... basidermal
of the ... second vertebra;
... ... salarium;
5, ...

Plate X, Figure 1: Photomicrograph of a frontal section of a _Pantosteus_ embryo at stage 5 (text, pp. 22-23); methylene blue and alizarin stains; 120 X. Sk, skull; T, _tripus_.

Plate X, Figure 2: Photomicrograph of a frontal section of a _Pantosteus_ embryo at stage 5 (text, pp. 22-23); methylene blue and alizarin stains; 120 X. DR2, dorsal rib of the second vertebra; PT, posterior process of the _tripus_; Sk, skull; Tr. T, transformator process of the _tripus_.


Sk
T


Sk
DR2
PT
Tr. T

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