DEVELOPMENT OF THE BOVINE SKULL

by

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INTRODUCTION

The formation of the mammalian skull has received some attention in the past but detailed attention only in the human. The work that has been done was restricted to specific aspects of development, such as the formation of the chondrocranium, times of appearance of ossification centers, and contributions of somitic components to the formation of the skull. Only in the human is there information pertaining to all 3 of these aspects. Information on other species is restricted to narrow phases of development with no overall description of skull formation.

Work dealing with the development of the human chondrocranium was reported by Keibal and Mall (1912), Nelson (1953), Arey (1965), Weichert (1965), Hunter (1936), Patten (1953), and DeBeer (1937). Hunter (1936) and DeBeer (1937) reported that the number of somites involved in formation of the occipital bone varies in different species from 3 to 5. Work dealing with ossification of the skull has been done principally with potassium hydroxide cleared and alizarin red stained embryos (Mall, 1906; Keibal and Mall, 1912; Noback, 1943, 1944; and Noback and Robertson, 1951).

This study was initiated to extend the morphological studies of the bovine skeleton to include the complete developmental story including ossification, supplementing the work by Hirt (1969) on the development of the appendicular skeleton and other studies on organogenesis in the bovine supervised by H. T. Gier in the Embryology Laboratory, Division of Biology, Kansas State University.

LITERATURE REVIEW

The earliest indication of skull formation in the human is a mass of dense mesenchyme which envelops the cranial end of the notochord during the 5th and 6th weeks (Arey, 1965). The sheath of mesenchyme, the meninx primitiva, becomes divided into the endomeninx and ectomeninx. The former is thin and vascular and closely surrounds the neural tube, the latter is stout and fibrous and its outermost layer gives rise to the perichondrium according to DeBeer (1937). When cartilage is initially forming, mesenchymal cells withdraw their processes, assume a rounded appearance, and become closely aggregated as the precartilage stage. Pre-cartilage masses become transformed into cartilage by development of intercellular substances. Cartilage grows both peripherally and interstitially. Peripheral growth of cartilage occurs by the inner cells of the perichondrium transforming into chondroblasts, causing the deposition of intercellular material. Interstitial growth occurs by deposition of intercellular substance which separates the chondroblasts from each other so the mass of cartilage grows as a whole (Nelson, 1953).

Development of the chondrocranium of the human begins in the second month of gestation and almost complete differentiation is achieved by the end of the third month (Keibal and Mall, 1912). Nelson (1953) divided the chondrocranium into 3 regions: 1) basal plate area, 2) trabecular or prechordal plate area, and 3) ethmoidal plate area. Chondrification begins during the seventh week medially in the occipital and sphenoidal regions (Arey, 1965). According to DeBeer (1937) and Weichert (1965) chondrification begins with a pair of flat curved cartilages, the parachordal plates, which flank the notochord on either side. At this time each inner ear becomes invested with an otic capsule (Arey, 1965). The parachordal plates extend laterally as far as the otic capsules and posteriorly to the point where Cranial Nerve X (Cn X) emerges (Weichert, 1965). The otic capsules eventually unite with the sphenoidal and occipital cartilages (Arey, 1965).

Posterior to this point there are two to four occipital vertebrae with which the parachordal plates later fuse during incorporation into the skull (Weichert, 1965). The occipital region of the skull has long been known to contain a somitic derivative. According to Hunter (1936), Chiaruge (1890) defined the occipital region as "a fragment of the trunk which is fused with that which is in front of it and which has modified its characters in a manner such that in the late stages of development and in the adult one cannot recognize the primitive condition".

According to Hunter (1936), Gegenbauer (1872), Froriep (1882), and Furbinger (1897) considered the skull to be made up of two regions: an anterior unsegmented region and a posterior segmented region. Both Froriep and Furbinger considered the vagus nerve to be the anterior boundary of segmentation. The number of myotomes present, the number of hypoglossal nerve roots, and the development of sclerotomes into occipital cartilage have all been considered as evidence for the number of occipital segments involved in formation of the occipital bone. Froriep reported three occipital myotomes in sheep (1882), and three in cow (1886); Mall (1891) reported three in man. Froriep and Mall reported three hypoglossal nerve roots in ruminants and in man respectively (Hunter, 1936). According to Singh Roy (1967), Froriep reported that four fused vertebral equivalents formed the occipital region in the ox, and DeBeer (1937) reported 5 occipital somites in "mammals". Houston (1967) reported four hypoglossal nerve roots in the dog which would indicate 5 somites in the occipital cartilage.

In regard to the most anterior somite, according to Hunter (1936), Williams (1910), Rex (1910) and Butcher (1929) reported that the somite anterior to the first somitic cleft breaks up without differentiating. Hunter (1936) reported that, in the rabbit, the posterior end of the unsegmented mesoderm anterior to the first intersomitic cleft does not exhibit a structure well developed enough to be called a somite. This somite was referred to as the "rudimentary somite" (Hunter, 1936). The parachordal plates grow larger than the occipital portions which fuse in the midline around the notochord, forming the basilar plate (Arey, 1965).

Anterior to the parachordals, as described by DeBeer (1937) and Weichert (1965), a pair of cartilages, the prechordals or trabeculae cranii, differentiate. The anterior ends of the prechordals fuse, forming the ethmoid plate, which grows anteriorly as the rostrum and later contributes to the formation of the internasal septum between the nasal capsules. Posteriorly, the prechordal cartilages unite with the parachordal cartilages leaving a prominent opening in the center, the hypophysial fenestra. At the same time the parachordal cartilages are developing, three pairs of capsules make their appearance about the developing olfactory, auditory and optic sense organs, which supposedly form the basis for the side walls of the chondocranium.

The chondrocranium is confined chiefly to the base of the skull whereas the sides and the roof are at this period a layer of connective tissue. In the human, the chondrocranium is then a unified cartilagenous mass (Arey, 1965).

In the chondrocranium, it is apparent that there is a confluence of primordial parts which will later become the bases of separate bones. Not until the various ossification centers are established in the chondrocranium is there anything but suggestions offered by the general shapes and positions of some parts of cartilage to determine the primordia of a specific bone. Many bones of the mammalian skull are composite, having been formed by the fusion of bones that were originally separate in lower animals. In such bones the ossification centers are multiple, since separate centers tend to appear in regions representing bones that were originally independent (Patten, 1953).

According to DeBeer (1937), Decker reported in 1883 on 2 stages of the chondrocranium of Bos and Fawcett (1918) modeled 2 stages in its development. No systematic study has been made of the development of the osteocranium (DeBeer, 1937).

Osteogenesis

Noback (1943) described three periods of development in the morphogenesis of bone: 1) period of differentiation, during which ossification centers appear (influenced by sex, heritable traits, nutrition, species, pathology and parity); 2) period of proliferation, which occurs at a constant rate and does not reach definitive proportions until birth; and 3) period of construction during which each bone is reorganized and grows in length.

Bone cells are of mesodermal origin. Once they have formed osteoblasts they produce collagenous fibers and deposit the ground substance of bone. As interstitial substance surrounds osteoblasts, they become osteocytes. In embryonic life, bone arises by differentiation of mesenchymal cells into osteoblasts (Maclean and Urist, 1967). Bones of the skull exhibit two types of bone formation, endochondral and intramembranous (Patten, 1953).

Endochondral bone formation occurs in most of the bones of the skeleton during embryonic life. The cartilage models are initially composed of hyaline cartilage, relatively free from glycogen and phosphatase and from enzyne systems concerned in glycolysis (Maclean and Urist,1967). Some realignment of cartilage cells takes place, the vascular buds from the inner layer of the perichondrium start eroding cartilage and forming primary marrow cavities (Nelson, 1953). Some of these connective tissue cells become osteoblasts and convert the calcified matrix to bone. As ossification begins, the cartilage cells are either arranged in longitudinal rows or are ungrouped, resting parallel to each other (Dodds, 1932). During this time the hypertrophic-cartilage cells accumulate glycogen, and glycolytic enzymes and alkaline phosphatase appear during the ingrowth of vascular mesenchyme from the periostium or perichondrium (Maclean and Urist, 1967). Hypertrophic cartilage cells also have high levels of glucose and ATP that may later be used in ossification (Kullman, 1970). This accumulation of glycogen and phosphatase are thought to bring about calcification (Hamm, 1969). Large multinucleate cells (chondroclasts) make their appearance and aid in the dissolution of cartilage (Nelson, 1953). Calcification also destroys cartilage cells (Hamm, 1969).

Intramembranous bone formation is seen in the formation of bones of the calvarium. In places where bone is about to appear, the intercellular substance, known as <u>ossein</u>, come to surround osteogenic fibers which lie between fibroblasts cells. Later these ossein spicules become ossified by the action of osteoblasts differentiated from mesenchymal cells. The spicules are converted into bony columns or trabeculae by the formation of layers of compact bone lamellae around the original bony spicule enclosing some of the bone cells in the lacunar space during this process and form bone cells (Nelson, 1953). Alkaline phosphatase is abundant in mesenchymal cells before the spicules are organized and diminishes as calcification proceeds (Bevelander and Johnson, 1950). Hamm (1969) stated that the term intramembranous is unfortunate since no membrane is involved but rather a layer of osteoblasts differentiated from the mesenchyme is responsible for this type of ossification.

Since bone does not undergo interstitial growth, it must grow and maintain its proper shape by deposition of bone tissues at certain places and removal from others. The deposition of ground substance may be due to glycoprotein granules in the osteoblasts, while at sites of rapid bone resorption, bone cells are devoid of glycoprotein (Heller-Steinberg, 1951). The removal of bone has been considered to be caused by large multinucleate cells called osteoclasts. The osteoclast may contain as many as 15 or 20 nuclei, which resemble those of osteoblasts and osteocytes (Maclean and Urist, 1967).

The mode of action of osteoclasts and the extent of their power is only speculative. Phagocytosis is not the usual mode of action although particles of bone have appeared in the cytoplasm of active osteoclasts. Probably some type of lysis is involved. Osteoclasts, probably secretory in function, have been observed with pseudopodia surrounding cartilage and bone cells (Dodds, 1932). The osteoclast may play a dual function in bone resorption: ingestion and degradation of mineralized debris, and release of secretory substances which may effect the solubilization of the collagenous matrix (Scott, 1967). As resorption occurs, osteoclasts may be left behind stranded in the marrow tissue (Arey, 1920).

Ossification of the human chondrocranium begins early in the third month but some membrane bones have begun ossifying before this (Arey, 1965). These membrane bones make up the dermatocranium (Patten, 1953). By the end of the third month in the human, the parietal, frontal, nasal and lachrymal bones, the maxilla, the zygomaticum, the squama temporalis, the tympanicum, the laminae mediales of the pterygoid process of the sphenoid, the vomer and the palatine bones are all beginning to become ossified as membrane bones (Keibel and Mall, 1912). Most primary ossification centers appear before the end of the fourth month. The sequence of appearance of the ossification centers of the skull during the first five months is 1) facial and calvarial centers, 2) basicranial centers, and 3) hyoid centers (Noback and Robertson, 1951).

<u>Mandible</u> has one intramembraneous ossification center. The cartilagenous bar of Meckel's Cartilage does not ossify, but membrane bone is substituted. The replacing bone develops ventrally in the base of the future jaw (Arey, 1965). In the human, on the 75th day, the jaw is 10mm long and has grown to

meet its fellow at the symphysis (Mall, 1906).

Maxilla arises from two centers of ossification (Patten, 1953): one forms the pre-maxillary and the other forms the main body of the bone. The pre-maxillary and maxillary are united along the alveolar border on the 56th day (Mall, 1906).

Occipital has four endochondral ossification centers: a median center ventral to the foramen magnum (basioccipital); a median center, which in early stages is quadripartite, rostral to the foramen (supraoccipital); and a pair of centers on either side of the foramen (exoccipitals) (Patten, 1953). In the human, by the 75th day, all the bones of the foramen magnum are united into the single tabular part of the occipital bone (Mall, 1906).

Zygomatic bone arises from a single center (Arey, 1965). The zygomatic (or malar) bone appears as a three cornered center just beneath and on the lateral side of the eye on the 56th day (Mall, 1906).

Temporal appears on the 56th day as a hook-like center representing mostly the zygomatic process. The primary and only center of the squamo-zygomatic enlarges, the zygomatic process growing larger and the squamous portion spreading out over the temporal region of the head (Mall, 1906).

Frontal appears as paired centers on the 56th day (Arey, 1965). On the 58th day, it is 8 mm in diameter, on the 73rd day, 10 mm (Mall, 1906).

Parietal arises from double ossification centers appearing in the human at 56 days as a delicate reticular nucleus about 3 mm in diameter and later apreading toward the occipital bone (Mall, 1906).

Sphenoid bone shows five pairs of endochondral centers: a pair in the small wings (orbitosphenoids); a pair in the greater wings (alisphenoids); a pair in the more rostral part of the body of the sphenoid (basisphenoid); and a pair in the lingulae (Patten, 1953). On the 83rd day the orbitosphenoid is

TABLE I

TIME OF OSSIFICATION OF SKULL BONES IN THE HUMAN (from Mall, 1906).

Bone	Ossification Centers	Gestation Day
Mandible	1	39
Maxilla	1	39-42
Pre-maxillary	1	39-42
Supraoccipital	1	56
Temporal	1	56
Interparietal	1	57
Basioccipital	1 (median)	65
Zygomatic	1	56
Squamo-zygomatic	1	56
Typanic ring	1	65
Frontal	2	56
Parietal	2	56
Pterygoid		57
Alisphenoid	1	58
Orbitosphenoid	1	83
Basisphenoid	1 (median)	83
Palate	1	57
Vomer	1	58
Nasal	1	57
Lachrymal	1	85

present as a rectangular bone. The basisphenoid at 83 days appears as two fairly large granules of bone on either side. The alisphenoid grows rapidly and reaches the frontal and temporal on the 105th day (Mall, 1906).

Ethmoid consists of medial and lateral parts. At four months endochondral ossification begins in lateral parts (Patten, 1953).

Palate Bone develops from a center of ossification on each side (Arey, 1965 On the 57th day the horizontal and vertical parts of the palate bone may be seen (Mall, 1906).

Vomer appears on the 58th day as two centers each 3 mm long, on the 65th day they are no longer, but are united at a single point near their anterior end. The union spreads rapidly throughout the length of the bone, and on the 73rd day it appears as a single groove-like bone 4 mm long (Mall, 1906).

Nasal bone is present on the 57th day and is well marked on the 65th day. It grows slowly, from 1.5 mm square on the 83rd day, to 2 mm on the 105th day (Mall, 1906).

Lachrymal bone is the last bone to appear in the head, on the 83rd day (Mall, 1906).

A review of the reported specific times of development, position of ossification centers, and time and type of fusion of separate bones of the skull clearly shows lack of completeness, lack of detailed descriptions, and lack of understanding of what happens and how it happens.

MATERIALS AND METHODS

The embryos and fetuses used in this study have been collected over the past 20 years from cows with known reproductive history, supplemented with some unknown specimens from local abattoires. Ages for specimens of unknown history were determined by use of a growth chart compiled from embryos of known ages. Measurements were taken with calipers calibrated to tenths of a millimeter. Crown rump, hind foot, and head length were measured to determine the age of the specimens.

For the ossification study, several embryos and fetuses were cleared in KOH, stained with alizarin red S and cleared in glycerine. Embryos with crown rump less than 40mm (50 days), were cleared by immersion in a 1% KOH solution for approximately 5 days with daily changes of the solution, then alizarin red solution was added to the KOH solution until it took on a pale lavendar color. The KOH-alizarin red solution was maintained, with daily changes, until the ossified area appeared red. Larger embryos and fetuses with crown rump measurements over 40mm were cleared in 2-3% KOH solution for 10-12 days and then stained by the same procedure.

Histological materials were prepared from Bouins or formalin fixed embryos, dehydrated in isopropyl alcohol, embedded in paraffin and sectioned at 10, 12, or 15 μ. Sections were stained in Harris hematoxylin followed by Mallory Triple stain, or with Periodic Acid Schiff followed by Harris hematoxylin.

For convenience of study the development of the bovine skull was divided into 2 sections, the formation of the chondrocranium, and the formation of the osteocranium. The serially sectioned embryos up to 52 days were used to study the forming chondrocranium. Models of a 41 and a 52 day chondrocranium were made. The 41 day model was constructed from projections of every 10th section, or 80 mu intervals. The 52 day model was constructed from projection of every 16th section or 192 mu intervals. Models were photographed, labels were added to the photographs, and the resulting illustrations were rephotographed. As the chondrocranium was studied, chondrification centers were noted and cartilages were named with regard to the specific bone they precede. Specimens of progressive ages (Table 2) were followed to obtain the developmental story.

The osteocranium was studied from the aged series of alizarin red stained specimens and serially sectioned embryos 42 to 74 days in age. The development of the osteocranium was studied with regard to the times of appearance of ossification centers, the number of centers that constitute each bone in the skull and the type of ossification in each bone.

TABLE 2

EMBRYOS AND FETUSES STUDIED

No.	Gestation Days	Section Type Cross	Long.	Whole Stained
509	27		L	
426	28		L	
600-1	28	X		
385-4	29		L	
353-30	32		L	
327	32		L	
620-11	32		L	
353-28	32	X		
217	34		L	
414	34		L	
236	34	X		
353-34	35		L	
189	35		L	
141	36		L	
437-27	36	X		
430	37		L	
165	38	X		
163	39		L	
600-27	40	X		
600-3	41[a]		L	
169	41		L	
700-1	42			S
551	44	X		
195	44		L	
385-12	46		L	
700-2	46			S
144	52		L	
700-3	52			S
1019	52[a]		L	
700-4	56[a]			S
700-5	58			S
700-6	60			S
700-7	61			S
1014	64		L	
700-8	66			S
700-9	68			S
700-10	85			S
700-11	95			S

[a] wax models made from these specimens

OBSERVATIONS AND DISCUSSION

Chondrocranium

The earliest indication of skull formation in the bovine is the development of the basal plate in precartilage by the dedifferentiation of the 5 most anterior somites at 32 days gestation. Chondrocranium formation then proceeds from 9 separate centers: 1) a single somitic region or basal plate, 2) paired otic capsules, 3) paired mandibular cartilages, 4) ethmoidal plate center, 5) paired frontalorbitosphenoidal centers and, 6) a postsphenoid center. Chondrification centers of the bovine skull have not been previously reported, and differ in many respects from descriptions of other species.

Nelson (1953) divided the chondrocranium into 3 distinct regions: 1) the basal plate area, 2) a trabecular or prechordal plate area which later forms sphenoid cartilage, and 3) an ethmoidal plate area. The bovine chondrocranium can be divided into 5 distinct regions: the three described by Nelson plus two more: 1) a distinct frontal-orbitosphenoidal region, which has been reported in previous studies by DeBeer (1937) and, 2) an otic capsule region that Nelson (1953) included in the basal plate region. The centers of chondrification that are found in the bovine embryo are well defined as illustrated in serial-section models (Figs. 1 and 2). The basal plate is formed from material of the first 5 somites dedifferentiated into a single sclerotome around the notochord (Fig. 1). The trabecular or prechordal plate area is formed from the mesenchyme ventral to the forebrain and anterior to the notochord. The ethmoidal cartilage is formed from a center ventral and anterior to the tip of the telencephalon (Fig. 1). The otic capsule cartilage is formed from a center around the otocyst (Fig. 2).

Chondrification occurs by accumulation of intercellular matrix between the previously compact precartilage cells and progresses centrifugally from the center within the precartilage layer to the limit of the particular layer. A distinct perichondrium forms on the surface of the cartilage from the outermost layer of precartilage (Fig. 3).

(1) The Somitic or Basal Plate Center is the precursor of the occipital bone and develops from the sclerotomes of the 5 most anterior somites. The first occipital somite begins to dedifferentiate by 28 days development (Fig. 4). The other 4 occipital somites quickly follow so that by 32 days the sclerotomic material of the 5 somites has begun to diffuse anteriorly under the floor of the mylencephalon around the notochord (Fig. 5). Since the ganglion of the 10th cranial nerve lies directly anterior to the first occipital somite and there was an occipital ganglion in each of the first 4 intersomitic grooves at 28 days, the locations of the original 5 occipital somites are marked at 32 days by the 4 occipital ganglia which, according to Houston(1967), will later form the 11th cranial nerve (Fig. 6).

By 34 days the major portion of the occipital somites has formed a definite sclerotome around the notochord extending craniad to the posterior limit of the pituitary anlage and laterally under the brain forming a shallow trough-like basal plate, from the pituitary anlage to the 5th intersomitic groove, as determined by the position of the 5th ganglion posterior to the 10th cranial nerve (Fig. 7). The cells of the basal plate proliferate into precartilage by 35 days. There is no distinguishable difference in the precartilage mass from its lateral limit to the notochord in its center. At 39 days the basal plate has begun to differentiate into cartilage, with chondrification starting between the 4th occipital ganglion and the first cervical ganglion, then proceeding anteriorly around the notochord to the posterior limit of the pituitary (Fig. 8). At 44 days the notochord of the head is completely enveloped in "basal plate" cartilage (Fig. 9).

The sphenoid portion of the chondrocranium forms from 2 separate centers independent of the occipital cartilage (Figs. 1 and 9). The postsphenoid chondrifies from the mass of precartilage ventral and lateral to the pituitary enlage (Fig. 1). The presphenoid arises as a posterior extension of the ethmoid plate anterior to the pituitary anlage. The post-sphenoid cartilage becomes continuous with the presphenoid and basioccipital cartilages by 46 days (Fig. 10).

Lateral and dorsal to the basal plate proper, the supraoccipital and exoccipital cartilages form as extensions of the basioccipital cartilage by day 41, thus completing the occipital in cartilage (Fig. 11).

Contrary to Froriep (1886) as quoted by Singh Roy (1967) who reported that 3 hypoglossal nerve roots and 4 vertebral equivalents become incorporated into the occipital region, this study found 4 hypoglossal nerve roots and 5 somites are incorporated into the occipital region of the bovine skull (Figs. 5 and 6). No "parachordal plates" were found flanking the notochord in the basioccipital region as reported by Weichert (1965); rather, the basal plate developed from one mass of somite tissue about the notochord extending to the anterior limit of the future basioccipital (Fig. 9).

(2) Otic Capsules are established in precartilage by 34 days, and as cartilage by 39 days. They form specifically the so-called *petrous temporalis* portions of the temporal bones. That portion of the skull anterior and dorsal to the otic capsule which will be the *squama temporalis* is never chondrified.

The otic capsule forms around the otocyst, beginning as a proliferation of enchymal cells into a precartilage stage by 34 days (Fig. 12). By 44 days cartilage encloses semicircular canals and internal auditory canal and the capsule cartilage has extended ventromedially to the basal plate (Fig. 13). The otic cartilage attains its complete form by 46 days with the chondrification of the bulla tympanica and the pterygoid process (Fig. 14). Chondrification in the otic capsule of the bovine chondrocranium appears to follow rather closely what has been reported by Arey (1965) and Weichert(1965) to occur in human and other mammals.

(3) Mandibular Centers differentiation begins as a proliferation of mesenchymal cells in the precartilage stage at 32 days (Fig. 15). Each mandible chondrifies from one center appearing in the region of the coronoid process of the future bone at about 37 days. By 39 days the mandible has chondrified as a 30mu thick rod, and is completely formed in cartilage by the fusing of the rami at the symphysis by 44 days.

(4) Ethmoidal Plate chondrifies from a bilaterally symmetrical precartilage center anterior to the tip of the telencephalon and spreads posteriorly to the presphenoid region and anteriorly to the end of the nasal region by day 39 (Fig. 16). The ethmoid plate is entirely chondrified by 46 days including the presphenoid and the dorsal and ventral turbinate cartilages, all of which arise as extensions from the ethmoid plate (Fig. 17).

Weichert (1965) reported that the internasal septum is the last part of the ethmoid to chondrify but I found chondrification to begin in the septum area and then spread laterally and posteriorly to include the presphenoid, conchae, and the dorsal and ventral turbinate cartilages.

(5) Frontal-Orbitosphenoidal Centers (FOS) proliferate as a plate of

mesenchyme in the precartilage stage above the eye by 35 days (Fig. 18). By 40 days, chondrification has begun and the FOS spreads centrifugally until the bottom half of the future frontal bone region is formed in a cartilage model, then extends ventromedially through the orbit to join the presphenoid cartilage (Fig. 1). By 52 days the FOS is complete in cartilage and extends posteriorly joining the otic cartilage. The bottom half of the future parietal bone is modeled in a parietal plate cartilage (Fig. 2). The FOS is joined to the otic, ethmoid and presphenoid cartilages by 52 days so is a prominent part of the bovine chondrocranium (Fig. 2). The FOS was described by DeBeer (1937) who reported that the FOS was included in a model prepared by Fawcett in 1918.

(6) Postsphenoid Center chondrifies from a bilaterally symmetrical precartilage center, postero-lateral to the pituitary anlage, and separate from the presphenoid and basioccipital cartilages (Fig. 1 and 9). By 46 days the postsphenoid cartilage has fused with the presphenoid and basioccipital cartilages (Fig. 10).

Unified Chondrocranium

After the appearance of the various chondrification centers, the cartilages grow peripherally until they meet, then they anastomose. A perichondrium forms both peripherally and medially to the plate of cartilage but the cartilage sheet expands within the precartilage layer, so fusion of cartilages consists of the differentiating edge of one cartilage contacting a differentiating edge of another cartilage. The first of these fusions occurs by 40 days between the FOS cartilage and the presphenoid cartilage, followed by the fusion of the postsphenoid to presphenoid and occipital cartilages by

46 days (Figs. 1 and 10).

The otic capsules extend ventromedially and fuse with the basal plate and the exoccipital wings by 46 days forming the posterior half of the floor of the chondrocranium thus completing the chondrocranium (Fig. 2).

At 46 days the development of the bovine chondrocranium is basically comparable to what Decker (1883) found in the 40mm stage, as reported by DeBeer (1937).

The sides of the chondrocranium are completed by the development of a parietal plate formed by the outgrowths and fusions of the FOS and otic cartilages by 52 days (Fig. 2).

At this time the brain rests in a cartilagenous cradle that extends slightly above the middle of the cerebral hemispheres, well up alongside the mesencephalon and metencephalon, and completely encircles the posterior one third of the mylencephalon. Chondrification has attained its zenith and from this time on cartilage is progressively replaced by bone as the major constituent of the skull.

Osteocranium

In the formation of the bovine osteocranium three types of ossification were observed.

Endochondral ossification is involved in the ossification of the bovine chondrocranium except for the mandibular, FOS, and the parietal plate cartilages. The ossification takes place by invasion of the cartilage by osteoblasts from the encapsulating connective tissue. Just previous to ossification the cartilage cells undergo hypertrophy and large multinucleate chondroclasts appear (Fig. 19). As the osteoblasts invade, bone matrix is deposited (Fig. 19). By the end of the 4 month the ethmoid, otic bone, sphenoid and occipital bones have become ossified endochondrally. These same bones also ossify endochondrally in the human (Patten, 1953).

Achondral ossification or what has in the past been called "intramembranous" ossification occurs in the formation of most of the bones of the bovine skull. Since no real membrane is involved in this type of ossification, as stated by Hamm (1969), I think the term intramembranous is erroneous and chose to call this type of ossification achondral rather than intramembranous since the distinctive feature of this ossification type is the complete absence of a cartilage base. In this case ossification begins with a proliferation of mesenchymal cells in a precursor layer within which trabecular bone is formed from osteoblasts differentiated within the layer (Fig. 20). The following bones of the bovine skull ossify by achondral ossification: the nasals, maxillae, premaxillae, interparietals, squamae temporalis, zygomatics, pterygoids, vomer, lacrimals and the upper halves of the parietal and frontals.

These same bones also ossify by the same process in the human skull according to Keibal and Mall (1912). However they reported the entire frontal and parietal bones ossified by this process.

Exochondral ossification is involved in the ossification of the mandible and the lower halves of the frontal and parietal bones. This type of ossification is distinctive in that bone is laid down right next to cartilage and the cartilage maintains its integrity while ossification occurs. Later the cartilage degenerates but it is not invaded by osteoblasts (Fig. 27 and 28).

Bones of the Bovine Skull, in Order of Their Appearance

Mandibles by 42 days have begun to ossify exochondrally as the first bones in the skull. Each mandible ossifies from a single ossification center located in the horizontal portions of the rami of the future bones (Fig. 21). By 46 days the horizontal portions of the rami are fully ossified (Fig. 22). The vertical parts of the rami are fully ossified by 52 days (Fig. 23). By day 56 the articular angles, which include the mandibular notches and the coronoid processes, are ossified (Fig. 24). The mandibular cartilages do not ossify but bone forms ventral and lateral to them (Figs. 27 and 28). Then the cartilages degenerate by some other process rather than by direct invasion by bone as seen in endochondral ossification.

Arey (1965) reported that in the human the mandibular cartilages do not ossify. According to Mall (1906), ossification of the mandibles in the human begins by 39 days. In the bovine the mandibles, as in the human, do not ossify from the mandibular cartilages. The bovine mandibles begin ossification by 42 days which is 3 days later than in the human.

Maxillae each appear from a single center of ossification in the region of the facial tuberosities of the future bones by 46 days (Fig. 22). By 56 days the facial tuberosity, the facial crest and the maxillary recess of each maxila are ossified (Fig. 24). At 61 days the maxillae are completely formed (Fig. 25).

Patten (1953) reported that in the human skull each maxilla arises from a double center of ossification. Mall (1906) described the maxillae as beginning ossification at 39-42 days.

Frontal bones appear from one ossification center dorsal to each orbital fossa in the region of the supraorbital grooves of the adult bones at 45 days (Fig. 22). By 52 days ossification has spread into the dorsal walls of each orbital fossa and medially, almost to the midline (Fig. 23). At 56 days ossification has spread posteriorly almost to the parietal sutures on each side and medially to the midline (Fig. 24). The frontals have taken their basic form by 61 days (Fig. 25). The upper half of the frontals ossify achondrally while the lower halves ossify exochondrally outside of the FOS cartilages.

Similarly, Arey (1965) and Mall (1906) reported the frontal bones appear ossified at 56 days in the human skull and Arey (1965) reported that each frontal ossifies from paired centers.

Zygomatic or malar bones arise from achondral ossification centers on each side which appear by 46 days just beneath and lateral to the orbital fossae as the zygomatic processes (Fig. 30). By 52 days the sygomatic bones have grown both anteriorly and posteriorly below the orbits (Fig. 23). At 56 days they have extended anteriorly to join the lacrimal bones and posteriorly to nearly join zygomatic processes of the temporal bones (Fig. 24). By 80 days the bones have fused to form the zygomatic arches on each side.

Similarly, Mall (1906) reported that the zygomatic bones in the human begin ossification by 56 days as mostly the zygomatic processes just beneath and lateral to the orbits. Arey (1965) reported that the zygomatic bones each arise from a single center of ossification.

Temporal bones or what has been known as the squamae temporalis each develop from a single achondral ossification center in the zygomatic process region at 46 days (Fig. 30). By 52 days the temporals have grown anteriorly to a junction with the zygomatic processes of the zygomatic bones (Fig. 23).

Similarly, Mall (1906) reported the squamae temporalis to appear ossified from a single center by 56 days in the human.

Premaxillae bones ossify from one achondral center on each side, anterior to maxillae, present by 50 days (Fig. 23). By 61 days they have taken the form of the adult bone, with the palatine fissure present (Fig. 25).

Vomer bone appears from a single achondral center of ossification at 50 days ventral to the postero-ventral part of the nasal cavity just anterior to the presphenoid region (Fig. 32). By 68 days it has grown anteriorly to the region of the palatine process of the premaxillae.

According to Mall (1906) the vomer in the human develops from paired centers of ossification appearing by 58 days and uniting at their anterior ends by 73 days.

Lacrimal bones each appear from a single achondral center of ossification at 52 days in the antero-lateral portion of the orbital fossae (Fig. 23). By 56 days the lacrimal bones have taken their basic form and are fused to the zygomatic bones (Fig. 24).

According to Mall (1906) in the human skull the lacrimal bones are the last bone to ossify, at 85 days.

Nasal bones arise from a pair of achondral centers first detectable at 52 days in the posterior region of the future bones dorso-medial to the maxillae. By day 61 the nasal bone have reached their anterior limits directly posterior to the premaxillae (Fig. 25).

Mall (1906) reported that the nasal bones each begin to ossify from one center at 57 days in the human.

Parietal bones each ossify from a single center appearing in the angle between the frontal and temporal bones at 56 days (Fig. 24). By 61 days each parietal bone has attained the fundamental shape of the adult bone (Fig. 25). The bottom halves of the parietal bones ossify exochondrally outside of the dorsal regions of the FOS cartilages while the top halves of the parietal bones ossify achondrally above the FOS cartilages.

Mall (1906) described each parietal to arise from one center of ossification at 56 days in the human skull.

Palatine bones arise from two achondral centers appearing as the horizontal parts of the future bones of 52 days (Fig. 31). By 56 days the vertical part and the pterygoid process of each palatine bone have become ossified. The palatine bones are completely formed as the posterior one third of the hard palate by 61 days.

According to Arey (1965) the human palatine bones also develop from a pair of ossification centers. Mall (1906) reported that both the horizontal and vertical parts of each bone can be seen in the human by 57 days.

Pterygoid bones each ossify from one achondral center just medial to the pterygoid processes of the future sphenoid bone at 56 days.

Hyoid bones start endochondral ossification by 56 days (Fig. 24) near the center of each segment and are essentially complete by 120 days.

Otic bones, or what have been called the petrae temporalis, are bones all by themselves. Each develops from 2 endochondral ossification centers: the tympanic ring which appears by 56 days (Fig. 24), and a center in the body of the cartilagenous capsule of the inner ear by 114 days. The body of the bone and the tympanic ring are still separate at 114 days.

Mall (1906) reported the tympanic ring in the human is present by 65 days, but mentioned no other center.

Interparietal bones each ossify from one achondral center posterior and medial to the parietals by 60 days (Fig. 25).

Occipital bone arises from 4 endochondral centers of ossification. The 1st center appears by 53 days in the middle of the basicoccipital cartilage (Fig. 23) followed by another center in each occipital condyle region of the exoccipital cartilages at 56 days (Fig. 24). By 56 days the 4th center has appeared in the supraoccipital cartilage (Fig. 24). By 120 days the different regions of the occipital have come together and fused around the posterior end of the myelencephalon leaving a prominent opening in the center of the bone, the foramen magnum.

Patten (1953) reported these same ossification centers in the human occipital. According to Mall (1906) ossification begins in the basicoccipital by 65 days and in the supraoccipital by 56 days in the human skull and reported that the parts of the occipital bone have met and fused by the 75th days.

Sphenoid bone arises from 6 ossification centers: one in each orbitosphenoid, one in each alisphenoid, one in the presphenoid and one in the postsphenoid. By 56 days the orbitosphenoid has begun to ossify in the center of the orbit (Fig. 24), followed by the presphenoid center which appears by 60 days. The alisphenoid center appears by 61 days, projecting laterally into the temporal wings of the sphenoid. The postsphenoid center appears in the postphenoid cartilage by 66 days. As the bone grows, the various parts fuse, so the sphenoid becomes a single bone with bilaterally symetrical wings by 85 days (Fig. 26).

Patten (1953) reported 5 pairs of ossification centers in the human sphenoid bone. According to Mall (1906) in the human skull the alisphenoid begins ossification at 58 days, followed by the orbitosphenoid and basisphenoid at 83 days.

In the ossification of the bovine skull the bones of the face and the dome of the skull appear first, followed by ossification of the basicranial bones, the otic, sphenoid, occipital, and ethmoid.

TABLE 3

TIMES OF APPEARANCE OF OSSIFICATION AND OSSIFICATION TYPES IN THE BOVINE SKULL

Centers	Type of Ossification	Day development in which first ossification centers appear Bovine	Human
Mandibles	Ex[b]	42	39
Maxillae	A[c]	45	39-42
Frontals	A, Ex	45	56
Zygomatics (malars)	A	46	56
Squamae Temporalis	A	47	56
Premaxillae	A	50	39-42
Vomer	A	50	58
Lacrimals	A	52	85
Nasals	A	52	57
Parietals	A, Ex	52	56
Basioccipital	E[d]	53	65
Palatines	A	54	57
Pterygoids	A	56	57
Exoccipitals	E	56	--
Supraoccipital	E	56	56
Orbitosphenoids	E	56	83
Hyoids	E	56	--
Otics	E	56	65
Presphenoid	E	60	83
Interparietals	A	60	57
Alisphenoids	E	61	58
Postsphenoid	E	66	83
Ethmoid	E	90	--

a From Mall, 1906

b Exochondral

c Achondral

d Endochondral

GENERAL DISCUSSION

The bovine skull begins formation by 32 days with the dedifferentiation of the anterior somites, much as reported by Mall (1891) except that there are 5 somitic sclerotomes involved in the occipital cartilage of the bovine rather than the 3 reported by Mall. By 52 days the chondrocranium is formed and ossification is well established, again corresponding fairly well with the times reported by Mall (1906) for the human. By 120 days, all ossification centers in the skull of the bovine have been established, although many of the centers are yet well separated and sutures are not complete until after birth.

This study included over 35 serially sectioned bovine embryos and fetuses, 28 to 64 days of age, for the study of chondrocranium and osteocranium development, thus providing an adequate series that covered all stages of early cranial development. KOH and alizarin red stained fetuses were utilized extensively in the study of the bovine osteocranium. This technique made possible a check of number and time of appearance of ossification centers by a method other than examination of stained sections. Also alizarin red staining of whole specimens provides the possibility of getting a clear view of relationships, as pointed out by Noback (1943 and 1944) and Noback and Robertson (1951) for the study of early ossification in the human skeleton. There were no observable differences in times of appearances of ossification centers between the sectioned and the alizarin red stained specimens. Gardner (1956) reported only slight differences in the accuracy of the two techniques. Radiological studies for determining ossification times in fetuses were reported by both Hirt (1969) and Gardner (1956) as being less accurate for determining the onset of ossification than stained sections.

The formation of the basal plate is the first indication of skull formation in the bovine. By 32 days the anterior 5 somites have dedifferentiated and their sclerotomic portions are diffusing craniad about the notochord under the mylencephalon (Fig. 5 and 6). By 34 days the sclerotomes of these 5 somites have fused into a bilaterally symmetrical precartilage mass completely surrounding the notochord rather than the reported (Weichert 1965 and DeBeer 1937) parachordal plates that later fuse to form the basal plate in mammals. No parachordal plates were found in the bovine embryos; the diffusing sclerotomic masses of the occipital somites became confluent around the notochord concurrently with the anterior-posterior confluence of those somites, thus there were no "parachordal plates" even in the precartilage stage. Five occipital somites were found consistently to be involved in the occipital cartilage, as generalized by DeBeer (1937) for all mammals, and thus contradicts Froriep's 1886 report (Singh-Roy, 1967) of 4 occipital somites in the bovine skull.

The ethmoid plate chondrification centers in the bovine begin in the nasal septum area by 39 days development and spread laterally to include the nasal conchae and turbinate cartilages rather than beginning with chondrification of nasals capsules as has been generally accepted (Weichert, 1965).

The otic capsule centers in the bovine develop comparably to the development described by Arey (1965) for the human and Weichert (1965) for mammals in general.

Chondrification is quite rapid, as the first cartilage is visible at 32 days; the mandibular, FOS, ethmoid plate, postsphenoid and basal plate cartilages are fused into a basal chondrocranium by 41 days (Fig. 1), and by 52 days the otic capsules have fused to the basal plate and parietal cartilages thus completing the chondrocranium except for later expansion (Fig. 2).

Ossification begins long before the chondrocranium is complete, with mandibular ossification around the mandibular cartilages (exochondral ossification) by 41 days and most of the face and calvarium ossification centers (achondral ossification) established by 56 days (Fig. 24), at which time the chondrocranium is fully established. Exochondral portions of the frontal and parietal bones formed lateral to the comparable cartilages by ventral extensions of the original achondral ossification centers. Classical endochondral ossification begins within the original basal plate (occipital, sphenoid, ethmoid) after the achondral and exochondral centers are all active and the definitive bones of the face and calvarium are establiblished. The first endochondral center to appear is in the basioccipital cartilage followed by ossification in the exoccipitals, supraoccipitals, orbitosphenoids, otics, presphenoid, alisphenoid, postsphenoid and ethmoid cartilages. The last endochondral center to form is in the body of the otic cartilage at about 114 days, at which time the basal plate is completely ossified, and bony sutures have formed between the parietals and both frontals and occipitals. All of the original chondrocranium has been replaced by bone by about 140 days which is reasonably close to the time accepted for the human (Mall 1891 and Patten 1953).

The classification system for ossification of the bovine skull was established on the basis of the geographic relationship of the developing ossification to pre-existing cartilage.

Three types of ossification were observed: 1) Endochondral ossification in which bone invades and replaces pre-existing cartilage, 2) Achondral ossification in which bone is laid down by osteoblasts in areas devoid of any cartilage base and, 3) Exochondral ossification in which bone is deposited directly outside of cartilage without invading the pre-existing cartilage. Members of the K.S.U. Embryology labarotory have found that the use of such a classification system is extremely useful in understanding the relationships of ossification centers to cartilage, and at the same time corrects the *misinterpretations* on the old systems of classification (endochondral, intramembranous), as was so forcefully brought to the attention of histologists by Hamm (1969).

Since there has been so little detailed work on skull formation, this report represents a major contribution in the way of establishing a series of descriptions of skull developments that can ultimately provide us with a reasonable understanding of the processes involved. The close parallel of time and sequences of events in the bovine and human are striking, even though timing of events in the human leaves much to be desired.

ACKNOWLEDGEMENTS

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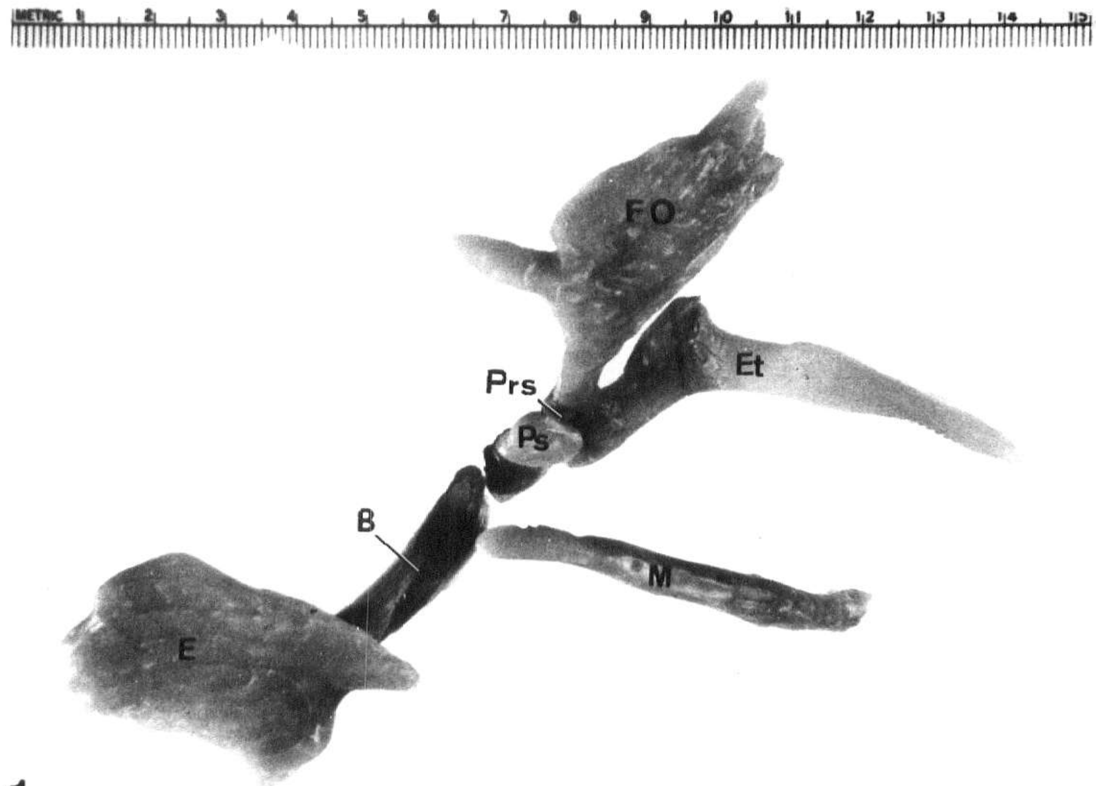
Also lastly, but not necessarily least, thanks are given to the night janitor, Mike Luse, whose friendly smiles and jokes kept me awake many a long and lonely night. Also thanks to Doris Wilson for her typing.

EXPLANATION OF FIGURES

Fig. 1. Wax model of one half of the bovine chondrocranium at 41 days development reconstructed from projections of every 10th section. The basioccipital (B), postsphenoid (Ps), presphenoid (Prs), and the ethmoid (Et) cartilages constitute the axis of the chondrocranium. The exoccipital (E) and frontal-orbitosphenoidal (FO) cartilages begin to establish the lateral walls of the cranium. The presphenoid and postsphenoid cartilages are yet unfused. The mandible (M) is completely formed in cartilage. (Slightly reduced from the original model, constructed at 25X)

Fig. 2. Wax model of one half of the bovine chondrocranium at 52 days as reconstructed from projections of every 16th section, or 192 mu intervals. The chondrocranium at this time has reached its zenith in development. All the different regions of the chondrocranium have appeared and are joined. The dorsal turbinate, ventral turbinate (Vt) and nasal conchae (NC) cartilages are formed as extensions of the ethmoid. The frontal-orbitosphenoidal and exoccipital cartilages are joined by the parietal plate (PP). The otic cartilage (OtB) is present and encloses the inner ear. The supraoccipital (S) and exoccipital (E) cartilages are developed as extensions of the basioccipital. The mandible is still independent of the chondrocranium proper. (Slightly reduced from the original which was constructed at 10X)



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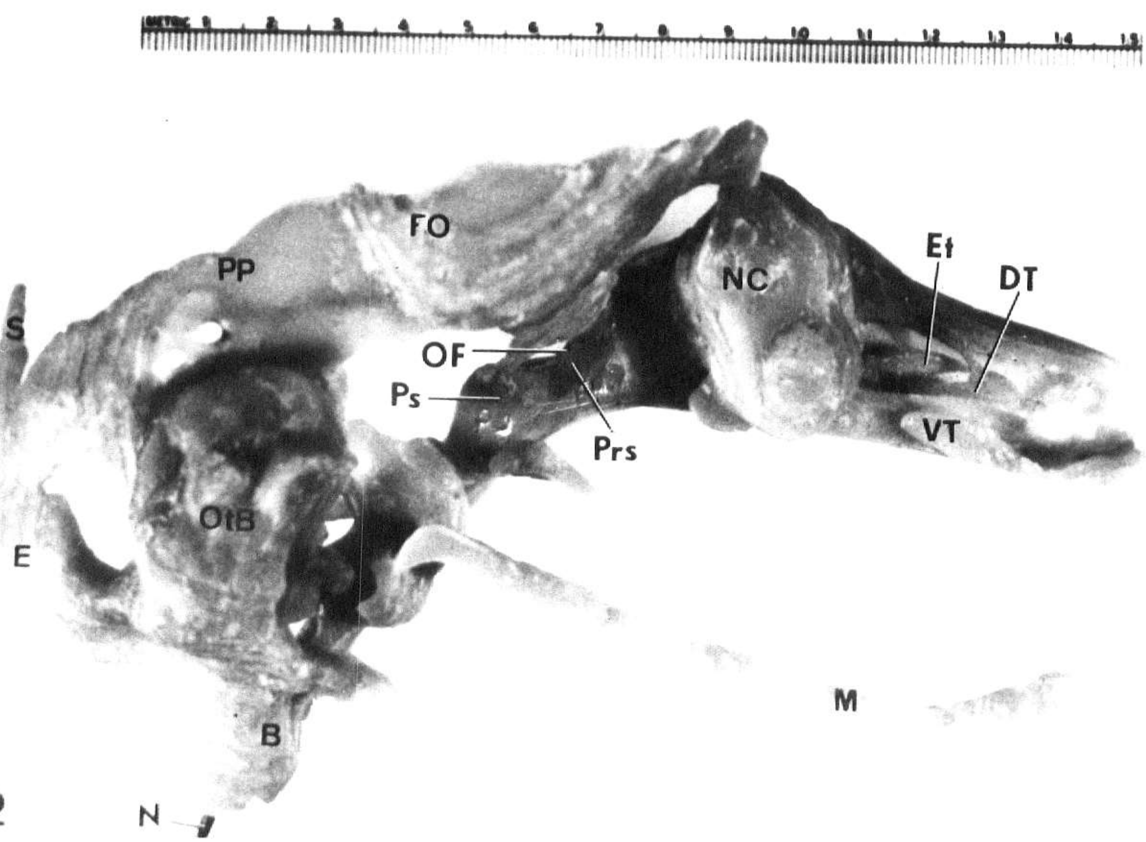

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EXPLANATION OF FIGURES

Fig. 3. Parasaggital section of basioccipital cartilage showing the transformation of mesodermal cells (Me) into precartilage (Pc) and cartilage (C). (PAS-HX, X120)

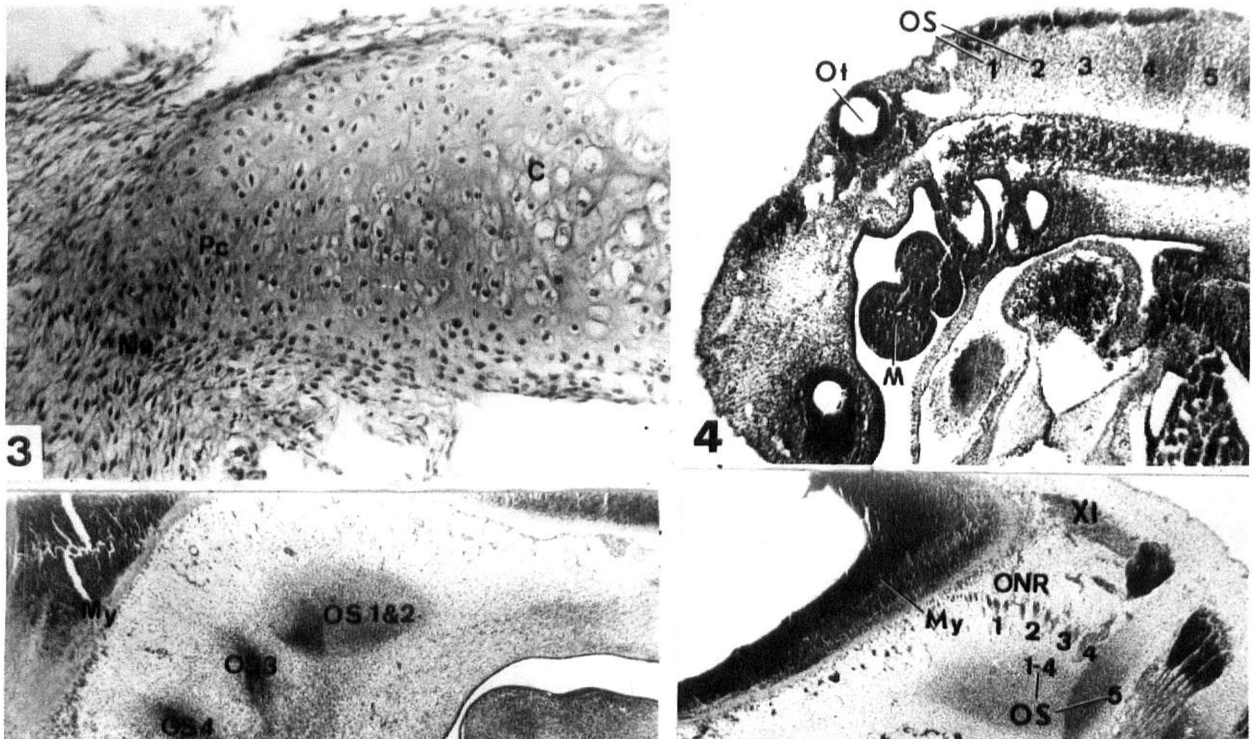
Fig. 4. Parasaggital section of a 28 day bovine embryo. The first 5 occipital somites (Os 1-5) are shown posterior to the otocyst (Ot). The first somite (Os 1) has begun dedifferentiation. (HX-Mallory triple, X30)

Fig. 5. Parasaggital section of a 32 day bovine embryonic head. The anterior occipital somite, anterior to the first cervical somite (CS) is dedifferentiating and diffusing anteriorly under the mylencephalon (My). (HX-Mallory triple, X30)

Fig. 6. Parasaggital section of a 32 day bovine embryonic head. The first five occipital somites are diffusing anteriorly under mylencephalon. Their original locations are marked by the 4 occipital nerve roots (ONR 1-4). (HX-Mallory triple, 15X)

Fig. 7. Median saggital section of a 34 day embryo head, the basal plate (BP) is formed in precartilage around the notochord (N), ventral to the metencephalon (Mt) and mylencephalon (My). The 1st cervical somite is the posterior limit of the basal plate. (HX-Mallory triple, 30X)

Fig. 8. Parasaggital section of a 39 day embryo head. Chondrification in the basal plate is proceeding anteriorly about the notochord from the 1st cervical somite to the posterior limit of the pituitary (P), forming a cartilagenous floor ventral to the metencephalon (M) and mylencephalon (My). (HX-Mallory triple, 40X)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EXPLANATION OF FIGURES

Fig. 9. Median saggital section of 44 day bovine embryo head. The head notochord (N) is completely enveloped by the basal plate (BP) cartilage and extends anteriorly from just anterior to the 1st cervical somite (CS) to the posterior level of the pituitary (P). The presphenoid (Prs) is present as a posterior extension of the ethmoid plate (Et) and is fused with the postsphenoid cartilage (Ps). The pituitary (P) is dorsal to the postsphenoid. (HX-acid fuchsin, X30)

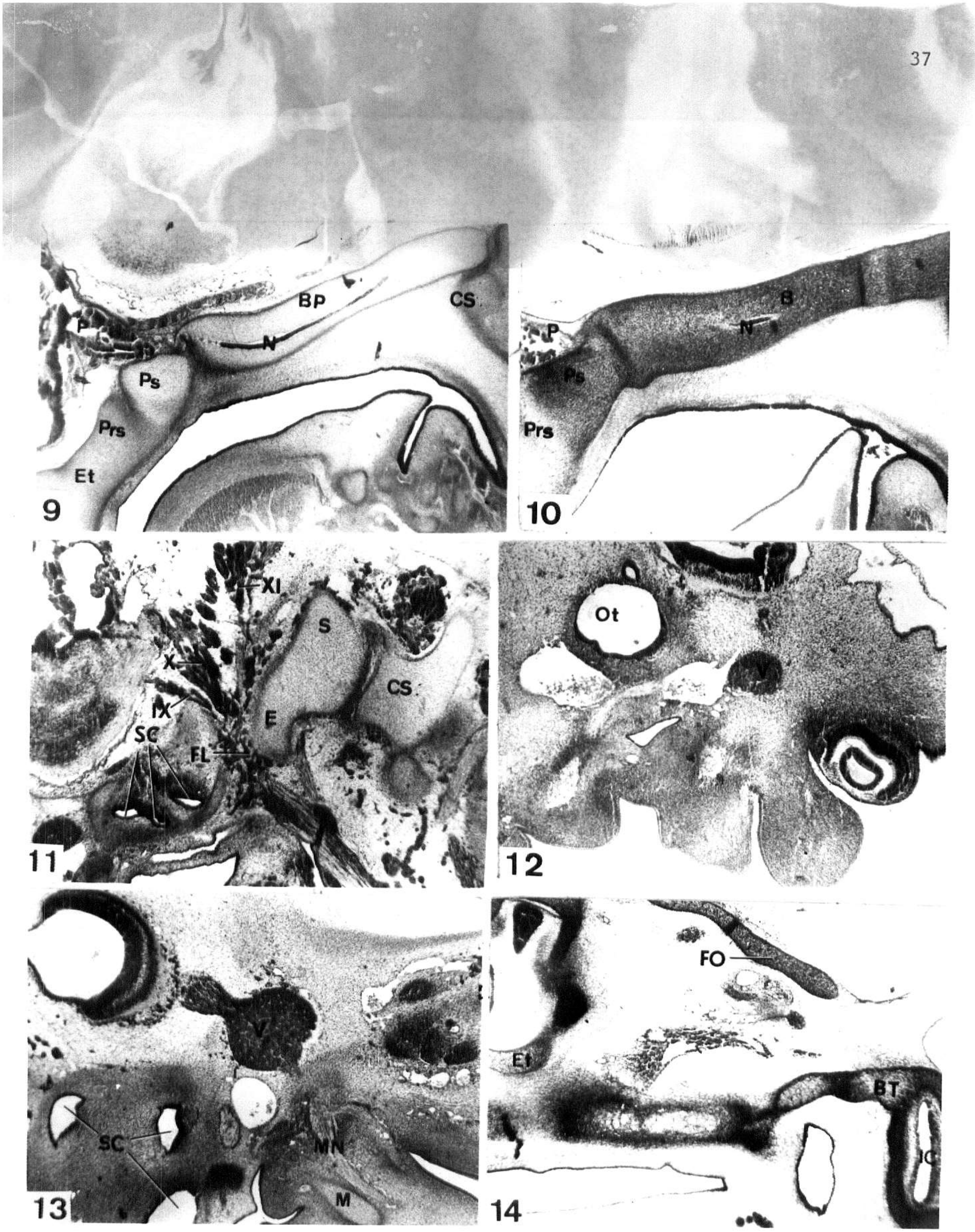
Fig. 10 Median saggital section of a 46 day bovine embryo head. The basilar portion of the basal plate or basioccipital (B) completely surrounds the notochord and is anastamosed to the postsphenoid cartilage just posterior and ventral to the pituitary (P). The presphenoid, post-sphenoid, and basioccipital cartilages are fused. (PAS-HX-X30)

Fig. 11. Parasaggital section of a 41 day bovine embryo head. The supra-occipital (S) and exoccipital (E) cartilages are formed as lateral and dorsal extensions of the basal plate and just anterior to the 1st cervical somite (CS). The roots of the glossopharyngeal (IX), the vagus (X) and the spinal acessory nerves (XI) are present and their nerves are exiting the foramen lacerum (FL). The semicircular canals (SC) are enveloped in the otic cartilage. (HX-Mallory triple, X30)

Fig. 12. Parasaggital section of a 35 day bovine embryo head. Precartilage is forming around the otocyst (Ot) which is posterior to the ganglion of the trigeminal nerve (V). (HX-acid fuchsin, X30)

Fig. 13. Parasaggital section of a 44 day bovine embryo head. The semi-circular canals (SC) are surrounded by cartilage and located posterior and ventral to the 5th cranial nerve. The mandibular cartilage (M) and mandibular nerve (MN) are present. (HX-acid fuchsin, X30)

Fig. 14. Parasaggital section of a 46 day old bovine head. The internal auditory canal (IC) is within the chondrified bulla tympanica (BT) and posterior and ventral to the ethmoid (Et) and frontal-orbito-sphenoidal (FO) cartilages. (PAS-HX-X30)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EXPLANATION OF FIGURES

Fig. 15. Parasaggital section of a 32 day old bovine embryo head. The mesoderm of the mandibular arch (M) is condensing into precartilage. The otocyst (Ot) is not yet surrounded by precartilage. (HX-Mallory triple, X30)

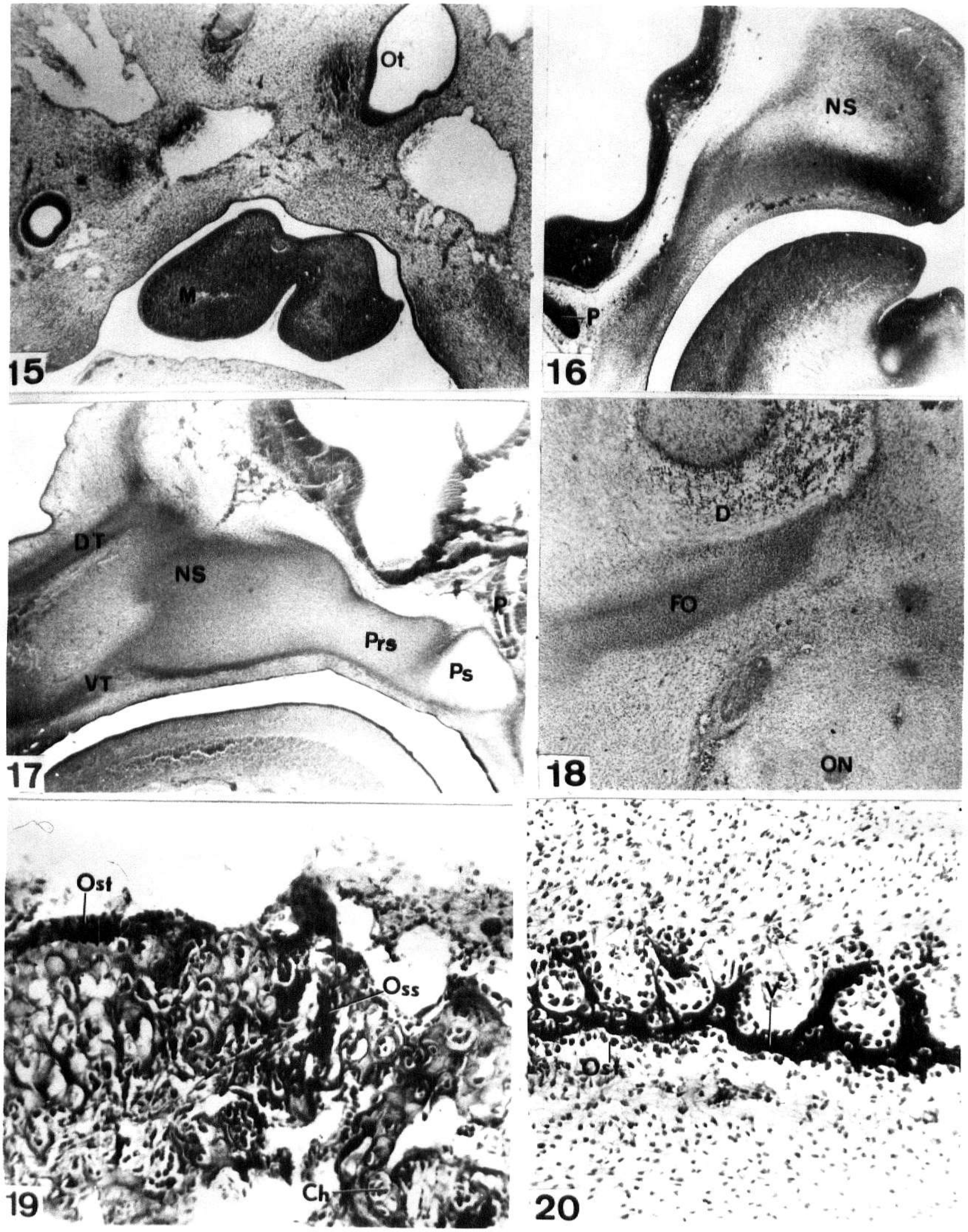
Fig. 16. Median saggital section of a 39 day bovine embryo, with the nasal septum (NS) of the ethmoidal plate chondrifying. The plate extends from just anterior to the pituitary (P) to the end of the nasal region. (HX-Mallory triple, X30)

Fig. 17. Median saggital section of a 46 day bovine embryo head. The ethmoid nasal septum (NS) is entirely chondrified including the dorsal turbinate (DT), ventral turbinate (VT) and the presphenoid cartilages. The presphenoid is fused to the postsphenoid (Ps) ventral to the pituitary. (HX-Mallory triple, X30)

Fig. 18. Parasaggital section of a 35 day bovine embryo head. The frontal-orbitosphenoid (FO) is in a precartilage stage ventral to the diencephalon (D) and dorsal to the optic nerve (ON). (HX-Mallory triple, X30)

Fig. 19. A section of the supraoccipital cartilage at 64 days. Osteoblasts are invading the cartilage and laying down bone (Oss) within the cartilage model. Large multinucleate chondroclasts (Ch) have appeared. (HX-Mallory triple, X120)

Fig. 20. A section of the vomer (V) ossifying achondrally showing the beginning of ossification by the osteoblasts (OST) within otherwise nondifferentiated mesenchyme. (PAS-HX, 80X)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EXPLANATION OF FIGURES

Fig. 21. Forty-two day KOH cleared, alizarin red stained bovine head. Ossification has begun in the ventral portion of the ramus of the mandible (M). Millimeter rule at edge of photo indicator magnification.

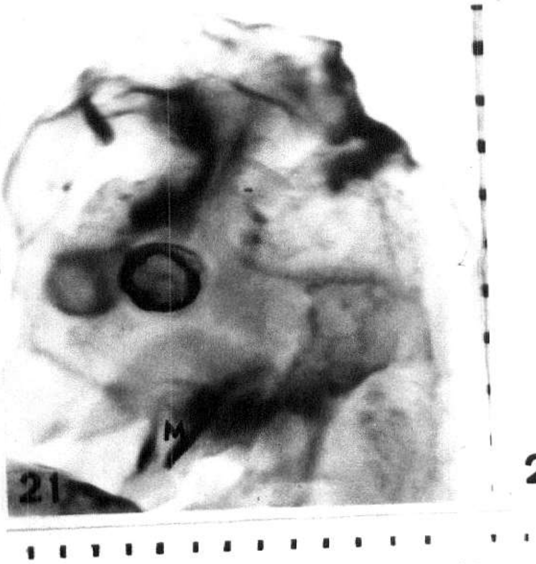
Fig. 22. Forty-six day KOH cleared, alizarin red stained bovine head. The mandible (M), maxilla (Mx), and frontal bones (F) have begun ossification.

Fig. 23. Fifty-two day KOH cleared, alizarin red stained bovine skull. The mandible, maxilla, frontal, premaxilla (Pm), temporal (T), basioccipital (B), lacrimal (L), nasal and vomer have begun ossification.

Fig. 24. Fifty-six day KOH cleared, alizarin red stained bovine skull. The exoccipital (E), basioccipital, hyoid (H), bulla tympanica (Bt), temporal, supraoccipital (S), parietal (P), frontal, orbitosphenoid (Ob), nasal, lacrimal, maxilla, premaxilla, zygomatic (Z), and mandible bone centers are ossified.

Fig. 25. Sixty-one day KOH cleared, alizarin red stained bovine skull. In addition to the bones that appear in the 56 day bovine embryo the interparietal bone (I) has begun ossification by 61 days.

Fig. 26 A median saggital view of an 85 day KOH cleared, alizarin red stained bovine skull, showing the postsphenoid, presphenoid, alisphenoid (Al) bones all of which have started to ossify after 56 days.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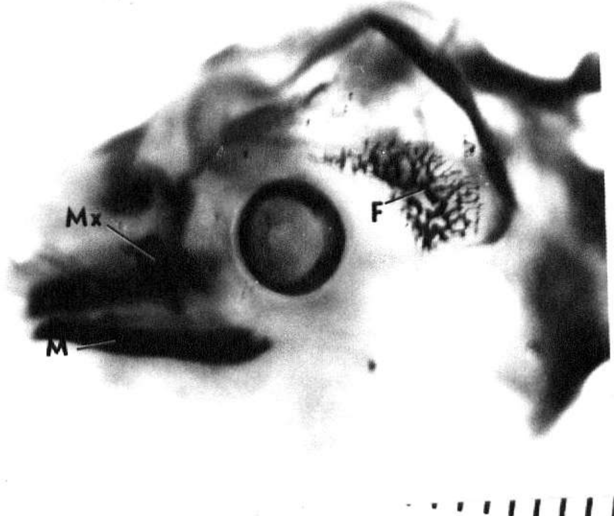
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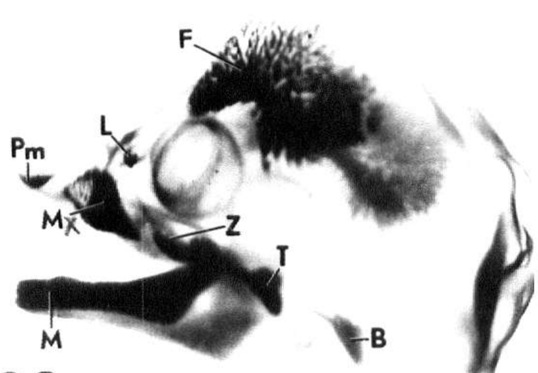
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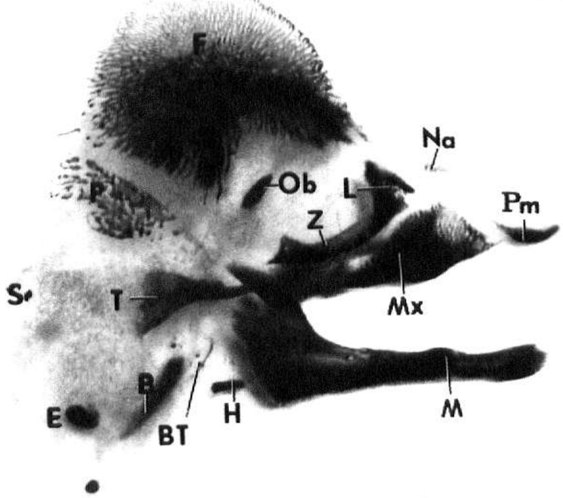
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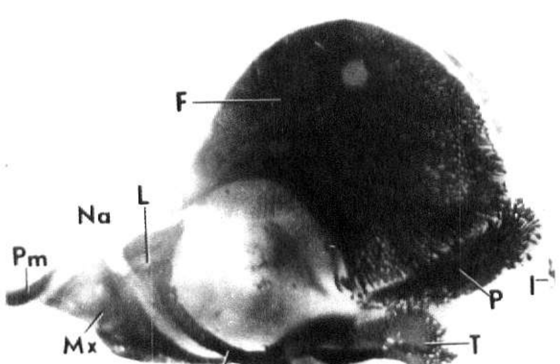
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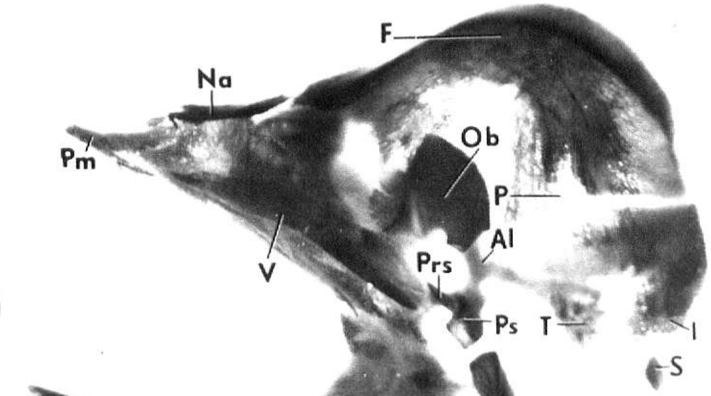
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EXPLANATION OF FIGURES

Fig. 27. Diagonal section of a mandible (M) of a 46 day bovine embryo showing exochondral ossification occuring ventrolaterally to the mandibular cartilage (MC). (PAS-HX-X25)

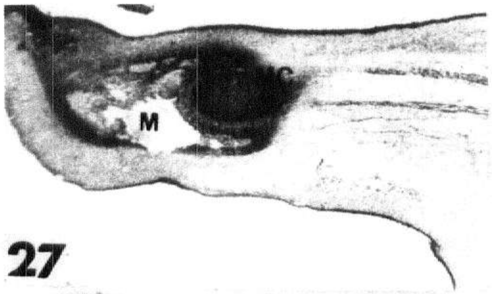
Fig. 28. Longitudinal section of a mandible of 46 day bovine embryo showing mandibular ossification (M) surrounding the mandibular cartilage (MC) but not destroying it. (PAS-HX-X150)

Fig. 29. Parasaggital section of a 46 day bovine embryo head showing the frontal (F) ossifying nonchondrally dorsal to the frontal-orbitosphenoidal cartilage (FO). (HX-Mallory triple, X30)

Fig. 30. Parasaggital section of a 46 day bovine embryo head. The zygomatic (Z) and temporal bones (T) are beginnign achondral ossification ventral to the frontal-orbitosphenoidal cartilage (FOS) and the optic nerve (ON). (HX-Mallory triple, X30)

Fig. 31. A parasaggital section of a 52 day bovine embryo head. The basioccipital (B) presphenoid (Prs), postsphenoid (Ps), nasal conchae (NC), ethmoid (Et) and mandibular cartilages are still present and ossification is occuring in the palatine (Pl), maxilla (Mx), premaxilla (Pm), and mandible. (HX-Mallory triple, X30)

Fig. 32 A median saggital section of a 52 day bovine embryo skull showing in addition to all the cartilages in Fig. 31 the ventral turbinate (VT) and dorsal turbinate (DT) cartilages. Ossification is progressing in the vomer (V), the mandible and the premaxilla. (HX-Mallory triple, X20)



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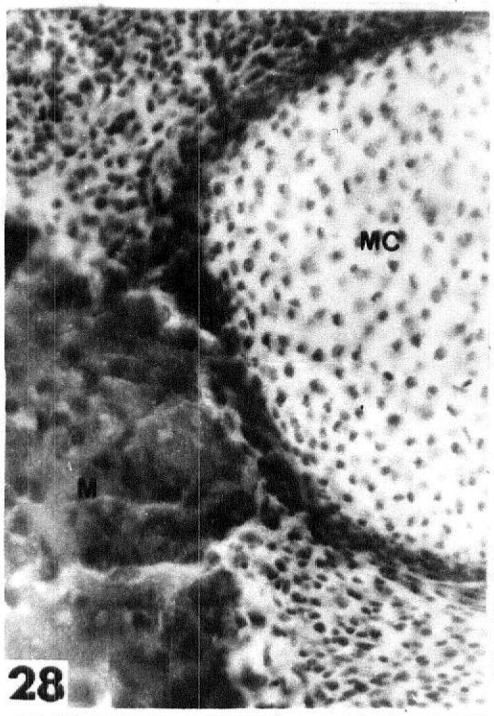

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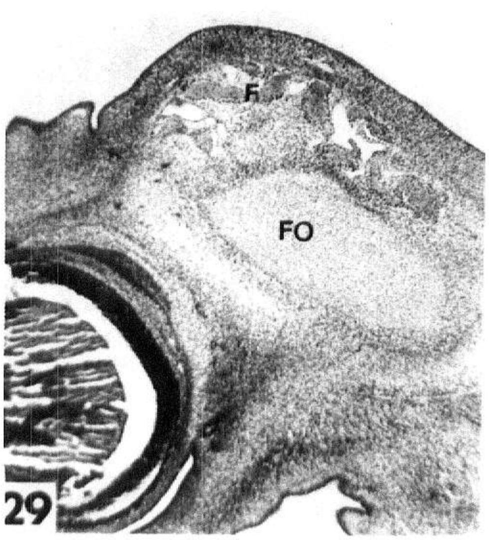

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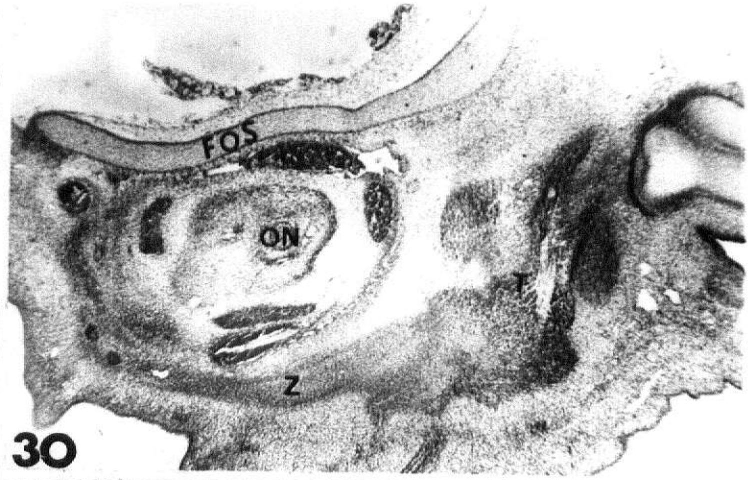

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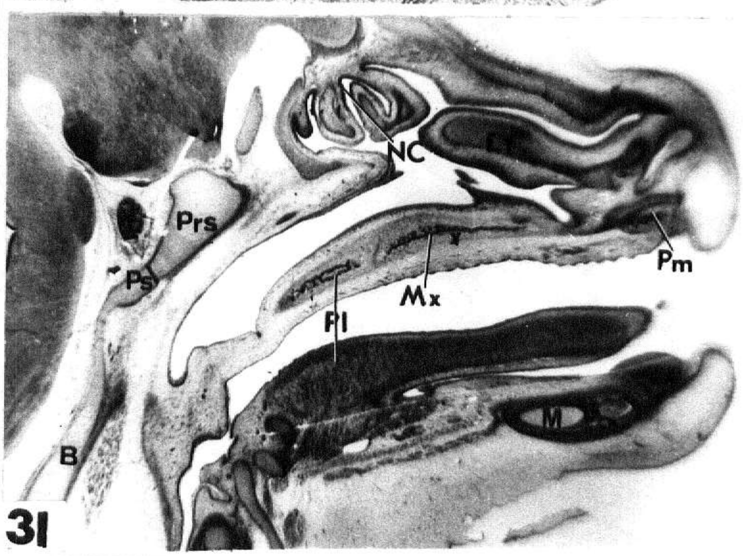

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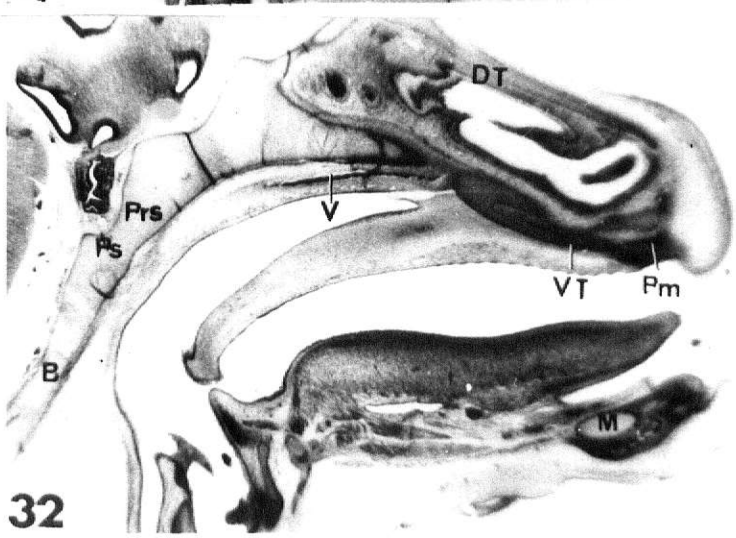

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DEVELOPMENT OF THE BOVINE SKULL

by

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AN ABSTRACT OF A MASTER'S THESIS

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ABSTRACT

Development of the skull in any mammal other than the human has received little attention. The only reported work on the bovine skull deals with the formation of the chondrocranium with no study on development of the osteocranium yet reported. This study was undertaken to remedy the inadequate studies on the bovine skull development and to supplement other work in our laboratory on bovine organogenesis. Over 40 serially sectioned embryos and fetuses and 14 alizarin red stained specimens were studied.

In bovine skull development, chondrification begins in the basal plate by 34 days; by 41 days the somitic portion of the basal plate, the otic capsules, the mandibles, ethmoidal plate, the frontal-orbitosphenoids and the postsphenoid of the chondrocranium are present and fusing so that the chondrocranium has taken its basic form.

By 42 days ossification has begun in the mandibles. At 52 days no ossification has taken place in the chondrocranium but other bones of the osteocranium have begun ossifying achondrally and exochondrally. At this time the mandibles, frontals, lacrimals, temporals, zygomatics, and the vomer have begun ossification.

The processes of chondrification and ossification occur concurrently from day 42 until day 53 when the chondrocranium has reached its height in development and ossification is beginning in the basioccipital cartilage. The chondrocranium is steadily destroyed by invading bone except for the mandibular, parietal plate and FOS cartilages which degenerate by other processes.

By 56 days the hyoids, basioccipital, exoccipitals, orbitosphenoids and otic capsules have begun ossification; also the parietals, palatines, ptergoids, and nasals have begun ossification. By 61 days these bones have

taken on the fundamental shape of their adult bone counterparts.

Achondral and Exochondral ossification centers are all established by 60 days. By 120 days the ethmoid and turbinate cartilages have begun ossification, completing establishment of the ossification centers.

In the bovine skull the bones of the face and the dome of the skull appear first, followed by ossification of chondrocranium as has been reported for the human skull by Noback and Robertson.

This study has confirmed, at least in the bovine, the generalization made by DeBeer for all mammals that 5 occipital somites are involved in the occipital bone and contradicts the generally accepted 3 or 4 somites in the occipital. Also in the bovine skull the age of the embryo at time of appearance of chondrification centers and extent of cartilage formation plus the time of appearance of ossification centers has been determined, most of which are slightly earlier than are found in comparable bones in the human.