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Development of The Cartilage and Musculature of The Human Larynx

by

H. Chris Wilson

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

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Abstract

Investigations into the development of the skeleton and intrinsic musculature of the human larynx demonstrated that these structures were derived from an inner and outer pharyngeal mesodermal condensation.

The sequence of skeletal development follows the reverse of what one would expect from phylogeny (see Negus, 1962), that is the cricoid and thyroid cartilages develop first followed by the development of the arytenoid cartilages. The thyroid cartilage develops from four portions, two on either side of the midline that later fuse and later still the halves on either side of the midline fuse. The cricoid develops unilaterally from before backwards and from below up whereas the arytenoid cartilages develop bilaterally with the vocal processes being the last portion to chondrify. The process of chondrification follows four stages. The dense mesenchymal stage exists from about 11.0 to 20.0mm. and the precartilage stage from about 17.0 to 22.5mm. The prochondral stage begins about 20mm. and continues beyond the 40mm. stage. The onset of the final stage of development (chondral) is sometime prior to 71.0mm. but lack of the necessary specimens prevents pinpointing the exact time.

The sequence of muscular development proceeds in a cranial to caudal direction. The interarytenoid primordium was first to develop followed by the posterior cricoarytenoid primordia which was in turn followed by the

simultaneous development of the thyroarytenoid mass and the cricothyroid anlage. All of the intrinsic laryngeal muscles develop from the inner mesodermal condensation with the exception of the cricothyroid which develops from the outer pharyngeal condensation. Myogenesis can be divided into four stages based on cellular appearence. The onset of the premyoblast stage was recognized at 11.0mm. and continued up until 20mm. The myoblastic stage ranged from about 17 to about 65mm. from 40 to about 65mm. The myotube stage commences prior to the 71.0mm. specimen (SH106). The fourth stage of myogenesis (myofiber) was not represented in the age groups of this study.

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I. Introduction

The investigation into the development of the cartilage and intrinsic musculature of the human larynx was the purpose of this study. Earlier studies of this structure have been conducted by Kallius (1897), Frazer (1910), Lisser (1911), Hast (1970) and most recently by Muller et al. (1981). Investigations on this subject however are by no means complete or without controversy. The developmental process by and large is still uncertain (Muller et al., 1981).

Much of the uncertainty surrounding the subject stems from the fact that a large collection of human embryos and fetuses is difficult to obtain and furthermore reconstruction of models from these specimens is very time consuming. Together these problems have tended to confine the limits of previous studies and have resulted in interpretations from incomplete data thereby promoting controversy in the literature. Ideally, further studies such as this one using a larger specimen collection must be undertaken in order to give a complete and comprehensive picture of the developmental process of the larynx.

A. Need for Reconstruction

Three dimensional reconstructions of a two dimensional object (the sectioned embryo or fetus) enables one to interpret the morphology of the object as it appeared prior to sectioning. This interpretation can in no way be achieved

by means of two dimensional microscopic observation prior to reconstruction. Realization of the third dimension is the single most important criterion in the interpretation of developmental anatomy and is inherent in the reconstruction technique. Reconstruction of a number of specimens in a series of pertinent ages is the most effective technique in achieving a comprehensive and detailed study of the dynamic developmental process.

Histologic examination of the sections following reconstruction enables one to see detail that can be masked in the reconstruction and, in addition, allows comparison of a number of similar aged specimens to those reconstructed. It follows then that both reconstruction and microscopic observation should be employed to assess the developmental process of this study.

B. Adult Morphology of the Larynx

The skeleton of the larynx is composed of the thyroid, cricoid and paired arytenoid hyaline cartilages. Two additional pairs of small elastic cartilages the corniculate and cuneiform are located at the arytenoid apices and within the aryepiglottic folds respectively. The epiglottic cartilage, although not considered in this study, is nonetheless a component of the larynx insomuch as its base is attached to the thyroid cartilage. The epiglottis functions in swallowing and to a lesser extent in the sensation of taste.

Movement within the larynx is made possible by the paired synovial cricoarytenoid and cricothyroid joints. The thyroid cartilage rotates through a horizontal axis placed between the joints concerned whereas the arytenoid cartilages both slide and rotate on an almost vertical axis placed through the cricoid (Gardner et al, 1975).

The intrinsic musculature of the larynx consists of paired cricothyroid, lateral and posterior cricoarytenoid, thyroarytenoid and vocalis muscles and the singular interarytenoid muscle. The latter is often differentiated into a deep transverse layer and a superficial oblique layer that is continuous with the aryepiglottic muscle (Gardner et al, 1975).

Motor innervation to the larynx from the nucleus ambiguus is carried to the superior and inferior laryngeal nerves via the tenth cranial nerve. The superior laryngeal nerves bifurcate prior to reaching the larynx into the internal and external laryngeal nerves. The internal laryngeal nerves pierce the thyrohyoid membrane to enter the larynx while the external (as the name implies) remains external to the larynx. The recurrent nerves recur around the subclavian artery on the right and around the aorta on the left. Both recurrent nerves enter the larynx posterior to the cricothyroid articulation as the inferior laryngeal nerves. The internal laryngeal nerve provides sensory innervation from the epiglottis to just above the vocal folds. The external laryngeal nerve supplies the motor

innervation to the cricothyroid muscle. The inferior laryngeal nerve carries the sensory innervation of the vocal folds and the region below plus the motor innervation to the remaining intrinsic musculature (Gardner et al, 1975).

The blood supply to the larynx comes from the laryngeal branches of the superior and inferior thyroid arteries (Gardner et al, 1975). These branches follow the pathway of the internal and inferior nerves. Blood is drained from the larynx by the superior, middle (if present), and inferior thyroid veins (Gardner et al, 1975). The superior thyroid veins are tributaries of the internal jugular veins while the inferior thyroid veins are tributaries of the brachiocephalic veins.

C. Laryngeal Cell Origin

The cell origin of the musculoskeletal system of the larynx develops from both *in situ* visceral mesoderm and from mesectoderm. The mesectoderm is derived from the cranial crest, that is, the neural crest cells from levels above the otic vesicles (Bronner-Frazer and Cohen, 1980). The neural crest cells that are induced by the endoderm of the laryngopharynx differentiate into the laryngeal cartilages (Bronner-Frazer and Cohen, 1980). The intrinsic musculature of the larynx is derived from *in situ* mesoderm and from the cranial crest cells (Douarin, 1980).

II. Materials and Methods

A. Specimens

All the specimens used in this study were human. The embryos and fetuses are from the University of Alberta's Anatomy Department (R.F. Shaner Collection; H. Zaw-Tun curator). All specimens in the Shaner collection are referred to by their code (SH) followed by the appropriate number (eg. 101). The serial sections are listed using letters and or numbers to denote first the slide number (eg. 22), then the row number (eg. 4), and finally the section number (eg. 5). The above example would then appear as SH101 22.4.5 in the Shaner system. The paraffin embedded specimens were serially sectioned in the transverse plane, stained with hematoxylin and eosin and mounted using standard histologic techniques. A list of the specimens and relevant data pertaining to them can be found in Table 1. The fertilization age of these specimens were calculated using the scale shown in Figure 1 which was adapted from Patten, 1968. It must be stressed here that these ages are approximations because the amount of spinal curvature varies from specimen to specimen and can therefore skew the Crown Rump (CR) measurement.

Six specimens (SH51, SH81, SH101, SH105, SH106 and SH108) were chosen for reconstruction. Each specimen was reconstructed first to show the cartilage and secondly to show the muscle attached to the cartilage. These specimens

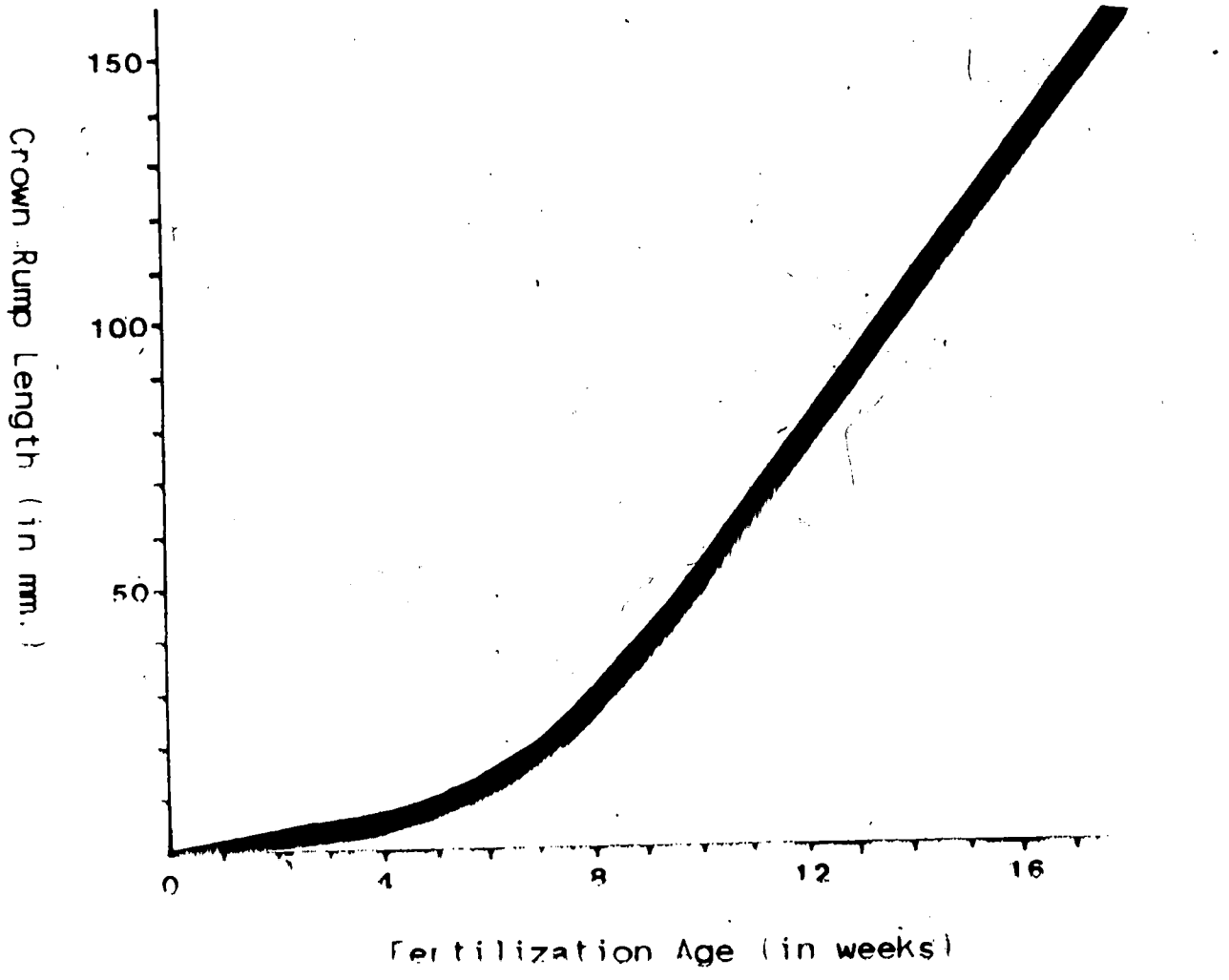
were chosen on the basis of their age and on the basis of their histologic quality. The criteria for quality were minimal tissue imperfections, and minimal obliquity in the plane of section, and, most importantly, the completeness of the serial sections. The latter quality is most beneficial when reconstructing to prevent distortion of the model. The ages were chosen at appropriate intervals (to the best of the collections limitations) to illustrate the dynamic process of the study. The largest specimen available in the collection was 110mm. (SH108). Therefore this study traces the development from its onset up until the fourteenth week.

Serial tissue sections of the remaining specimens listed in Table 1 were analyzed using a microscope. This provided a comparison to those specimens that were reconstructed and also provided intermediates between those reconstructed.

It is appropriate here to point out (as Lisser did in 1911) that reproduction of developing tissues presents peculiar difficulties. These difficulties stem from the fact that transition zones of tissues exist at the various developmental stages. For example, clear demarcation of these zones in developing cartilage presents problems when tracing the tissue image for the reconstruction. The line between precartilage and cartilage is not distinct but rather a case of overlapping zones. Therefore a decision must be made as to where the best fit line shall be drawn. An attempt to control this source of error in the method can

be done by using similar judgement criteria throughout the reconstructions.

Figure 1 Age Estimation from Crown Rump Length



Adapted from Patten, 1968

B. Magnification Technique

Reconstructions must be magnified else they provide little if any knowledge beyond gross dissection. When magnifying a specimen certain parameters must be delt with, such as the tissue section thickness, the thickness of the reconstruction media, and the size to which one wishes to enlarge the reconstruction

The thickness of both the tissue section and the reconstruction media limit the magnification factor that can be incorporated into the reconstruction. For example in this study the wax plate media used is on the average 1500 micrometers thick. If the tissue section thickness is 10 micrometers then 1500 divided by 10 is 150. Therefore if every tissue section is used in the reconstruction, then the image must be magnified 150 times the size of the section. If a 50x enlargement is desired, then every third section must be used in the reconstruction and, likewise, if 75x is desired then every other section must be used and so on.

The accuracy of the reconstruction deminishes proportionately to the number of tissue sections deleted in the reconstruction. That is, a 150x model in the above example would be more accurate than the 50x model. From the data gathered here it is not recommended to delete more than [illegible] sections in the reconstruction technique

C. Reconstructions

Three dimensional reconstructions of the above mentioned specimens listed in Table 1 were made using the Born wax plate technique (see Gaunt and Gaunt, 1978). Dental wax plates (Kerr Flexo wax) were chosen as a modeling medium because they are relatively inexpensive, have consistent thickness, and are easily managed.

The first step of the Born technique is to produce magnified tracings of the enlarged specimen to be reconstructed. To perform this task, pencil tracings on plain white paper were drawn from serially projected images of the tissue sections. The Bausch and Lomb Tri-Simplex and the Leitz Prado microprojectors were used to project the tissue images.

The second task of this method is to transfer the tracings from the paper onto wax plates. This is accomplished by slipping the paper under the template and lightly etching the image onto the wax surface using a drafting pen stylus. The translucency of the wax plates allows accurate replication of the underlying template.

The wax plate is then cut along the etched line using a modified Adams C-920 microdissecting scalpel. The modification involved shortening the length of the blade bringing the handle and therefore the hand closer to the wax surface and thereby increasing the cutting precision.

Before the wax image can be removed from the surrounding wax the raised edges made by the cut must be

flattened. This is done by planing both surfaces of the wax plate with a razor blade held at 80 degrees. This procedure will create a greater surface tension when the plates are glued.

The wax plates are then removed from the surrounding wax images and serially stacked with glue intervening. The images were stacked using the guidance of reference points in the tissue sections (such as the notochord and aorta). Excessive glue that is squeezed out from between the wax sections should be removed before hardening to prevent this task from becoming cumbersome. The glue can be removed by guiding the cutting tool along the edge of the model.

After the glue has hardened a warmed spatula will smooth the jagged appearance that results from the slight uneveness of the external surface of the model. Flexible wires can be heated and inserted into the model at weak points if strengthening is required. Painting the model adds resilience and the use of colors adds contrast. A polymer medium added to the acrylic paint prevents this mixture from beading on the wax model.

The reconstructions of the laryngeal skeleton in this study included tissues in the precartilage, prochondral, or chondral stage of development. Dense mesenchyme, the precursor to precartilage, was omitted. Therefore the onset of chondrification was arbitrarily defined with the precartilage stage in this study.

A similar method was used in the study of the intrinsic musculature. The dense mesenchymal stages were omitted from the reconstructions and the onset of myogenesis was arbitrarily defined by the presence of myoblasts.

It would perhaps be useful here to briefly outline the stages of myogenesis proposed by Boyd (1960) and restated by Fischman (1972). The promyoblast is the primordial muscle cell that is indistinguishable from adjacent mesenchyme. The myoblast is the spindle-shaped uni or multinucleated cell that lacks myofibrils. The myotube is an elongate multinucleate cell that contains myofibrils only at its peripheral margins. The myofiber is the adult muscle cell characterized by peripheral nuclei and numerous myofibrils. Cytological studies of human fetal skeletal muscle demonstrated that the myofiber stage is not reached until 20 weeks (Tomanek and Colling-Saltin, 1977). Therefore the myofiber stage of differentiation was absent from the present study.

Although the above classification is useful, it has limitations in the present study. Section thickness of embryonic tissue does not allow suitable histological examination of the process of myogenesis (Muller et al. 1981). Nonetheless this classification was useful in providing guidelines for the description of embryonic muscle development. Similar to the above classification of muscle development, four stages can also be recognized in the development of cartilage. The first sign of developing

cartilage is the condensation of mesenchyme (dense mesenchymal stage). In the precartilage stage the tissue destined to become cartilage is organized into a template of a particular cartilage. In the prochondral stage chondroblasts are recognizable and the presence of chondrocytes is diagnostic of the chondral stage.

D. Tissue Section Analysis

In-depth tissue section analysis was undertaken, following the reconstruction period, on the specimens listed in Table 1. These observations allow relatively rapid assessment of a number of specimens in an attempt to quantify the data by increasing its sample size. Specimens of similar age to those reconstructed were used to confirm the observations of the reconstructed specimens. This is done because slight variations occur between specimens of equivalent age (see Muller et al. 1981). Specimens of intermediate age are used here to assess the stages between the models and therefore fill in any gaps that might exist in the developmental process.

E. Illustrations and Photography

The illustrations incorporated into the presentation of the data were meant to enhance the results section and thereby allow the reader to gain a better understanding of the topic. These illustrations represent both the cartilage and muscle reconstuctions. The photographs found in the

appendices represent key tissue sections that revealed detail not apparent in the reconstuctions or that were intermediate between the reconstuctions. They are listed in the appendices in the same order that they appear in Table 1. They are titled by their catalogue number and slide section number. In the text they will be referred to by their plate number and letter as presented in the list of photographic plates.

TABLE 1 Human Specimens Described in the Present Study

Catalogue #	Size (mm.CR)	Age	Fixative	Stain	ST
SH5	4.75	27days	Formalin	H and E	ns
SH42	6.0	29days	Formalin	H and E	10
SH28	7.0	30days	Formalin	H and E	10
SH21	8.5	32days	Formalin	H and E	12
SH40	10.0	33days	Formalin	H and E	10
SH45	11.0	34days	Formalin	H and E	10
SH43	12.5	35days	Formalin	H and E	10
SH11	14.3	37days	Formalin	H and E	ns
SH80	17.5	39days	Formalin	H and E	15
SH83	18.0	39days	Formalin	H and E	20
SH51	15.5	6.5wks.	Formalin	H and E	20
SH94	22.5	7.3wks	Formalin	H and E	20
SH81	23.5	7.5wks	Formalin	H and E	20
SH91	26.0	7.8wks	Formalin	H and E	25
SH98	26.0	7.8wks	Formalin	H and E	20
SH101	28.5	8.0wks	Formalin	H and E	20
SH105	40.0	9.0wks.	Bouins	H and E	10
SH106	71.0	11.5wks	Formalin	H and E	20
SH109	110.0	11.8wks	Formalin	H and E	ns

[illegible] specimen cut in transverse plane

[illegible] not stated

Abbreviations used in the Illustrations and Photographs

A... Arytenoid Cartilage

AE.. Aryepiglottic Muscle

Ap.. Arytenoid Primordium

C... Cricoid Cartilage

Cp.. Cricoid Primordium

Ct.. Cricothyroid Muscle

H... Hyoid Cartilage

Hp.. Hyoid Primordium

IM.. Inner Mesodermal Concentration

LCa. Lateral Cricoarytenoid Muscle

OAm. Oblique Arytenoid Muscle

Oh.. Omohyoid Muscle

OM.. Outer Mesodermal Concentration

PCa. Posterior Cricoarytenoid Muscle

Sh.. Sternohyoid Muscle

St.. Sternothyroid Muscle

T... Thyroid Cartilage

Ta.. Thyroarytenoid Muscle

TAm. Transverse Arytenoid Muscle

Tf.. Thyroid Foramen

Th.. Thyrohyoid Muscle

Tp.. Thyroid Primordium

UM.. Undifferentiated Mesoderm

V... Vocalis Muscle

III. Review of the Literature

As stated in the introduction previous studies concerning either or both the intrinsic musculature and cartilage of the larynx have been conducted. The research reviewed here are the major works of Frazer (1910), Lisser (1911), Hast (1970 and 1972) and Muller et al. (1981). The works of Kallius (1887), Strazza (1888), and Soulie and Bardier (1907) were reviewed by Muller et al. (1981) and Hast (1970). Pertinent information from these reviewed papers will be incorporated in this chapter and in the discussion. The works of Schaffer (1907), Goerttler (1954), Mumladze (1962), Rudan (1969), Giordano-Lanza and Marinelli (1969), Morgan (1970), and Krawczynski (1978) were unusable because of translation difficulties.

Frazer (1910)

The work of Frazer was, for the most part, concerned with the early development of the larynx. For a detailed study of the reinterpretaion of the origin and early development of the respiratory primordium, trachea and esophagus the reader is refered to Zaw Tun (1982). The work of Frazer included eight specimens (5.0mm., 6.6mm., 7.0mm., 8.5mm., 12.0mm., 16.0mm., 22.0mm., and 35.0mm.). He made three wax reconstructions that pertain to this study at 16mm (6weeks), 22mm (8weeks) and 35mm (9weeks).

The Laryngeal Cartilage

In the 16mm. specimen only the body of the hyoid was in the prochondral stage. All the laryngeal cartilages were in a condensed mesenchyme state. The cricoid and arytenoid anlages were continuous and the thyroid concentration was continuous with the greater horn of the hyoid.

By 22mm., chondrification was seen ventrally uniting the laminae of the cricoid cartilage giving it a U-shaped appearance. The arytenoid anlages had not changed from the dense mesenchymal state seen in the previous reconstruction. Two chondrification centers, a dorsal and a ventral, were present in each half of the thyroid primordium. Each thyroid ala (the ventral chondrification) was convex externally with the ventral border turned in. The two alae were further separated above than below and had not as yet fused ventrally.

The 35mm. cricoid anlage had grown enormously out of proportion in relation to the other laryngeal cartilages and was relatively complete except for a narrowed ventral junction. The arytenoid primordia had begun chondrification except at their inferior margin (vocal processes). The thyroid cartilage had increased in length and breadth. The inferior thyroid horn had made contact with the cricoid anlage and the alae were fused in the ventral midline except at the cranial margin. The inferior thyroid tubercles were distinct.

To summarize the above, the cricoid anlage was first discernable at six weeks and by seven weeks each half of the cricoid had chondrified below and in front. The two halves were united ventrally by a thin chondrified bridge of tissue. By eight weeks the chondrification of the cricoid was relatively complete. After presenting data that revealed the early cricoid developing in a unilateral U-shaped manner from before backwards, Frazer (1910) contradicted himself by stating "the commencement of the cricoid is evidently bilateral" (p183)

The arytenoid anlage was at first larger than the cricoid anlage but remained dense mesenchyme and grew little during the seventh week. By the eighth week chondrification had progressed throughout the arytenoid, except at the vocal processes. No joint cavities were present between the arytenoids and cricoid at this stage. The vocal processes did not chondrify until the third month.

The thyroid forms bilaterally with the two chondrification centers per side that meet at the thyroid foramina. Frazer (1910) stated that the chondrification began by the end of the first month.

Laryngeal Musculature

Frazer (1910) described the origin and development of the intrinsic musculature of the larynx from an inner and outer constrictor layer. At 7mm two distinct layers were found surrounding the fifth arch mass but only an inner layer surrounded the fourth arch mass. This inner circular

layer was surrounded by an outer circular layer. He noted the difficulty in following the details of the transformation of these "constrictors" but stated the outer constrictor gave rise to the cricothyroid muscles and the inner constrictor gave rise to the remaining intrinsic musculature.

B. Lisser (1911)

The work of Lisser (1911) concentrated on the development of cartilage, muscles and nerves in six specimens. However only four specimens were described (10.5mm., 12.5mm., 16.0mm. and 20.0mm.). The two specimens not described (14.0mm. and 19.5mm.) were very similar to the 12.5mm. and the 20.0mm. specimens respectively and therefore Lisser felt no need, beyond stating their similarity, to include them in his description.

In the 10.5mm. specimen the laryngeal skeleton was purely dense mesencyme but the anlages of the skeletal components were discernible. The cricoid presented itself as a single ventral mass destined to become the ventral arcus. Lisser (1911) noted that at no time did he see the cricoid composed of two lateral halves not joined ventrally. Kallius' work (1898) also stated that chondrification was initiated in the ventral arcus. The arytenoid primordia were indefinite masses superior to the cricoid. The thyroid consisted of a mesenchymal 'horseshoe mass' without signs of superior or inferior horns.

Lisser (1911) described three muscle masses at this stage. An isolated posterior cricoarytenoid, a thyroarytenoid/lateral cricoarytenoid mass, and a cricothyroid. The presence of any arytenoid muscle mass was doubtful at this stage and there was no sign of the aryepiglotticus muscle. There was a slight continuity between the fibers of the inner laryngeal and outer pharyngeal masses. Lisser (1911) felt this continuity was greatly overemphasized in the literature.

The laryngeal skeleton at 12.5mm. was still in a precartilaginous state. The cricoid appeared similar to that found in the 10.5mm. specimen but was at a further advanced stage of condensation than that found in the thyroid anlage. The arytenoid anlage was apparent, distinct and had an oval shape. Except for the addition of the superior and inferior horn rudiments the thyroid was similar to the 10.5mm. condition.

The muscles in the 12.5mm. embryo were similar to the above although slightly more distinct. Lisser noted the first appearance of the transverse arytenoid muscles and stated a partial connection with that of the posterior cricoarytenoid muscle.

The laryngeal skeleton of the 16mm. embryo was precartilaginous but initial signs of chondrification were evident in the hyoid. The cricoid anlage had grown further in relative proportion to the thyroid which made it the most prominent of the laryngeal skeletal components during the

precartilage stage. The arytenoid anlages appeared not to have made any changes over that of the 12.5mm. condition The arytenoids seemed to be the slowest of the laryngeal skeletal components to develop. The two portions of each half of the thyroid had fused around the thyroid foramen but few changes had taken place over the previous condition

The muscles of the 16.0mm. specimen were further distinguished than in the previous states The cricothyroid, posterior cricoarytenoid, and the transverse arytenoid muscles were definitely isolated. The lateral cricothyroid and the thyroarytenoid were as distinct as they would appear in the adult.

By the onset of the 20.0mm. stage, the laryngeal skeleton was beginning to chondrify The lateral portions of the cricoid were cartilaginous but the ventral arcus connecting these portions remained precartilage. The arytenoids remained precartilaginous and, with the exception of the muscular processes, the shape was approaching the adult condition. The thyroid alae were united ventrally by condensed mesenchyme. The superior horn were continuous with the greater cornua of the hyoid by a mesenchymal bridge and the inferior horn was greatly exaggerated in size.

By the 20.0mm stage all the muscles were clearly differentiated It must be emphasized that Lisser (1911) did not describe the vocalis and he stated the aryepiglotticus at this stage was not definitely isolated from the interarytenoid muscle. Therefore his reference to all the

muscles excluded the vocalis and the aryepiglotticus, which is a continuation of the oblique arytenoid muscle.

As pointed out earlier Lisser (1911) contended too much emphasis had been placed on the early appearance of an inner and outer muscle mass and the latter differentiation of the laryngeal musculature. In his discussion he noted observations of a frontally sectioned 19.5mm. embryo that sheds light on this problem. The inner constrictor (sphincter laryngeus) does not have a horizontal direction to its fibers but rather a frontal direction to the fibers.

Lisser (1911) interpreted the laryngeal muscle development as consisting of groups of muscles that later differentiate. By stage 18 four muscle groups were discernable (cricothyroid, posterior cricoarytenoid thyrocricoarytenoid, and the interarytenoid masses) and by stage 21 the thyrocricoarytenoid mass differentiated into the thyroarytenoid and the lateral cricoarytenoid.

C. Hast (1970, 1972)

The work of Hast (1970) was for the most part a review of the literature. He did not reconstruct any specimens but he did make observations of the development of the muscles (1972) and cartilages (1970) from serial sections.

The first intrinsic muscle to develop, according to Hast (1970, 1972), was the interarytenoid which was discernable at about 9mm. Shortly thereafter, the posterior cricoarytenoid and [illegible] the lateral

cricoarytenoid anlages are apparent at about 11mm., whereas, by 13mm. both the cricothyroid and thyroarytenoid primordia can be recognized. Hast (1970, 1972) agreed with Frazer (1910) on the derivation of the intrinsic laryngeal musculature from an inner and outer "constrictor". He further contended that the downgrowth of the inferior horn of the thyroid separated the outer constrictor into an anterior portion (the cricothyroid) and a posterior portion (the inferior pharyngeal constrictor).

Hast (1970) went into more detail where the cartilages of the larynx were concerned. He stated that the thyroid and cricoid anlages appeared at about five weeks but that chondrification did not commence until the end of the seventh week. The arytenoid anlages began chondrification at the begining of the third month in the vocal processes and the development was completed in the vocal processes. The corniculate cartilages began chondrification towards the end of the third month whereas the epiglottic and cuneiform anlages began chondrification in the fifth and seventh months respectively.

Hast (1970) divided the development of the laryngeal skeleton into four stages: the mesenchymal stage (embryos 9-15mm.), the early prechondral stage (embryos 15-25mm.), the prechondral stage (specimens 25-55mm.), and finally the cartilaginous stage (fetuses 55mm.+).

Hast (1970) divided the development of the laryngeal

mesenchymal stage (4-6mm.), the inner and outer "constrictor" stage (7-9mm.), the early muscular differentiation stage (10-12.5mm.), and the stages in which the laryngeal muscles differentiate; four (13-15mm.), five (16-18mm.), and six (19-23mm.).

D. Muller, F., O Rahilly, R., and J.A. Tucker (1981)

The work of Muller et al. (1981) had two objectives. The first was to study the laryngeal musculature and its innervation at the end of the embryonic period proper because the development is still uncertain. The second was to explore the degree of variation that existed in the laryngeal musculature during the eighth week of embryonic development. Six specimens of the eight week period (27-32mmCR) were studied and of those five were reconstructed.

During the end of the embryonic period proper the cricoid consisted of an arch and two laminae that formed a continuous ring of cartilage. There were no synovial cavities present at this stage. The muscular processes of the arytenoids were cartilaginous but the vocal processes were mesenchyme. The thyroid consisted of two cartilaginous laminae continuous ventrally by a mesenchymal bridge. The superior horns may or may not have been continuous with the laminae and the inferior horns were variable in size among the six specimens. The oblique line denoting the attachment of the sternothyroid and the thyrohyoid was present. Neither

the corniculate nor the cuneiform cartilages were apparent.

It was noted that in two of the six specimens a twig from the external laryngeal nerve passed through the thyroid foramen but no blood vessels accompanied this nerve. The authors stated that this nerve probably anastomoses with the anterior branch of the inferior laryngeal nerve.

The muscles that were well distinguished at the end of the embryonic period proper included, the cricothyroid, posterior and lateral cricoarytenoid, and the thyroarytenoid. The aryepiglottic muscle had not yet formed but the vocalis was beginning to differentiate. Although the oblique arytenoid muscles were not mentioned in their discussion it is assumed that if the aryepiglottic muscles were present then the oblique arytenoid muscles must be also because of their continuity. The transverse arytenoid muscles were present.

In their discussion the authors point out that the laryngeal skeleton required important modifications in position, shape and relationships to attain the adult form. They also stated that variation within the same age class definitely existed. The variation noted in this study must be emphasized because it lends to the explanation of variable times noted with respect to the establishment of a particular developmental structure.

The following material represents the results of the present study. The development of the laryngeal skeleton will be presented, followed by the development of the laryngeal musculature. These in turn will be followed by the discussion.

IV. Development of Laryngeal Cartilage

Prior to the onset of laryngeal chondrification, the skeleton can be vaguely recognized as dense mesenchyme or precartilage. The thyroid anlage was discernable at 12.5mm.(SH43) as dense mesenchyme and by 14.3mm.(SH11) the ventral arcus of the cricoid was dense mesenchyme. At 17.5mm.(SH80) the body of the hyoid was prochondral and both the thyroid and cricoid had obtained a precartilage status. It was not until the 20mm. stage of development (SH51) that the arytenoid primordia presented themselves as dense mesenchyme early precartilage and by this time both the thyroid and [illegible] of development.

A. SH80 17.5mm.

The earliest signs of chondrification in the laryngeal skeleton was observed in this embryo. The precartilaginous thyroid anlage was represented by two paired segments on both the right and left sides of the laryngeal lumen. These paired segments were the superior horns and laminae of the thyroid. The superior horns (Plate 6A) were rod shaped with little depth. The thyroid laminae (Plate 6B) were pear shaped from above and taper inferiorly to a ridge shape. Again these segments had little depth.

The cricoid anlage was barely discernible (Plate 6C) as early precartilage. This was true of all sections containing the cricoid primordium. There was no evidence for the

prochondral state and no other laryngeal cartilages were discernable.

B. SH83 18.0mm.

The development of the laryngeal skeleton had made little advancement over that of SH80. The superior horns and laminae of the thyroid primordium were basically the same as observed in the preceeding specimen (Plates 7C and 8A). The cricoid anlage (Plates 8C) was more distinctly in aprecartilage stage of development. There was no evidence suggesting that the arytenoid primordia were in the precartilage state.

C. SH51 20mm.

The earliest stage chosen to reconstruct the laryngeal skeleton was that of SH51. Although catalogued as 15.5mm this embryo by comparison with other specimens was closer to the 20mm stage of development. More will be mentioned about this topic in the discussion. At this stage both the thyroid and cricoid anlages had begun to chondrify (early prochondral stage, Plate 10B). The arytenoid primordia were for the first time discernable as early precartilage (Plate 10D).

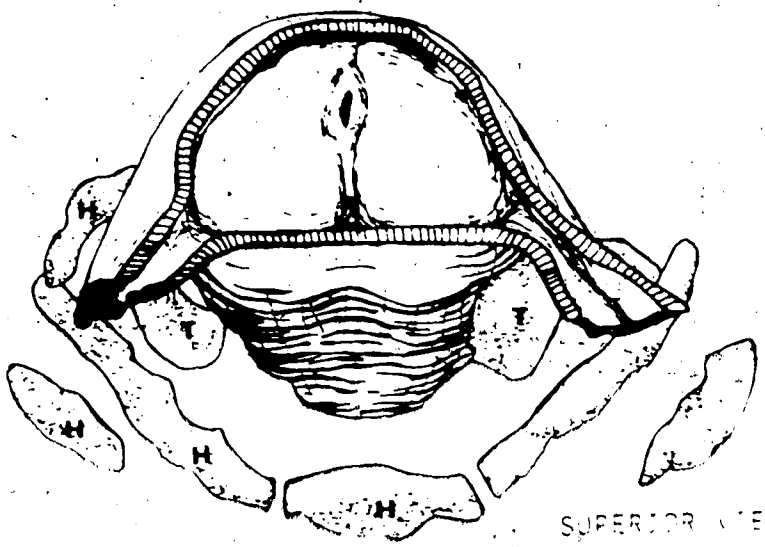
The thyroid anlage consisted of four segments, two on either side of the cricoid. The superior horns were continuous through a mesenchymal bridge with the greater cornua of the hyoid and positioned centrally over the

thyroid laminae. The laminae were situated ventral, lateral, and somewhat cranial to the cricoid. Each lamina from a superior view was somewhat concave and triangular in shape with the apex of the triangle slanted medially.

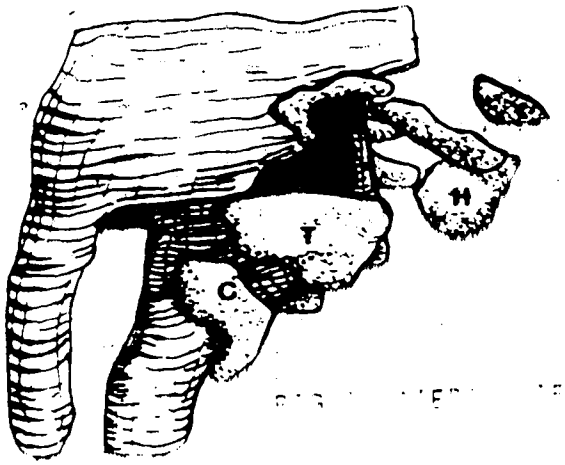
The cricoid anlage was a single U-shaped ventrally sloping mass encircling the anterior half of the laryngeal lumen. From an anterior view the uppermost border of the cricoid was adjacent to the midpoint of the thyroid laminae. From a lateral view the most anterior portion of the cricoid was subadjacent to the thyroid laminae midpoint and the posterior margin extended beyond the thyroid laminae.

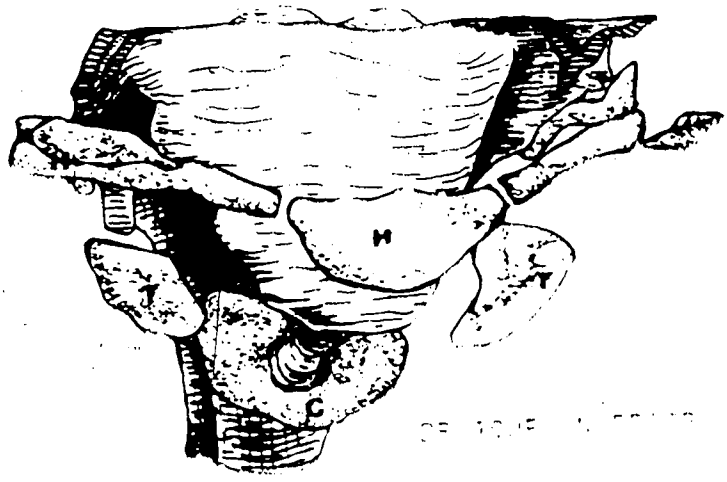
n SU94 22.5mm

By this stage of development both the cricoid and thyroid anlages were to be considered in the prochondral state. The ventral arcus of the cricoid (Plate 13C) was well developed at the anterior and most inferior margin of the cricoid. The arytenoid primordia (Plate 11B) were observed for the first time as an early prochondral stage and were semitriangular masses with little depth. Therefore between SU81 and SU94 the later precartilage stage of arytenoid



SUPERIOR VIEW





E. SH81 23.5mm.

This embryo was the second specimen to be reconstructed. Both the thyroid and cricoid anlages were prochondral and had doubled in size from the SH51 reconstruction. The fusiform arytenoid anlages were small in comparison to the other components of the laryngeal skeleton and may be considered in an early prochondral state (Plate 14B). Although the arytenoid anlages were not included in illustration 2 of this chapter, they were included in illustration 8 of the following chapter.

The superior horns had made contact with the thyroid laminae yet a patency persisted surrounding the fusion point. These patencies have been termed the thyroid foramina in the literature. From a lateral view the superior horns were somewhat cranial to the uppermost margin of the hyoid greater cornua and the inferior border of the laminae lay adjacent to the midpoint of the cricoid. The laminae were more concave than those seen in the previous specimens and the anterior inferior borders were approaching each other in the midline. The inferior horn was discernable for the first time but not very prominent. The superior horns were exaggerated in size.

Although the cricoid anlage had doubled in size over that of SH51, the relative growth of the laryngeal lumen kept pace. Therefore the ventral arcus of the cricoid did not appear to have increased its posterior growth to completely encircle the lumen. The uppermost border of the

cricoid was located adjacent to the midpoint of the thyroid laminae. The most anterior portion of the cricoid (the arcus) was approximately subadjacent to the anteriormost border of the thyroid. The posterior margin was approximately parallel to the posterior margin of the thyroid (Plate 16B).

The arytenoid primordia were situated superior to the uppermost level of the cricoid and were roughly fusiform in shape.

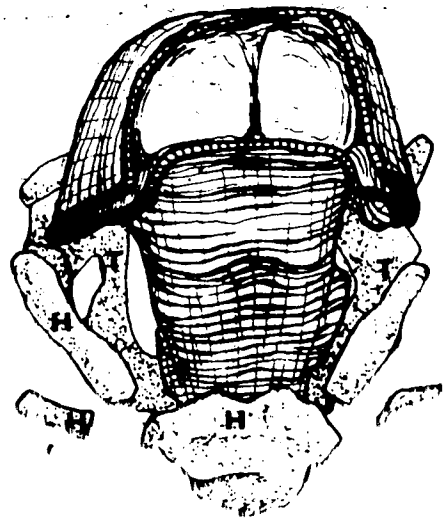
[illegible] 26.0mm.

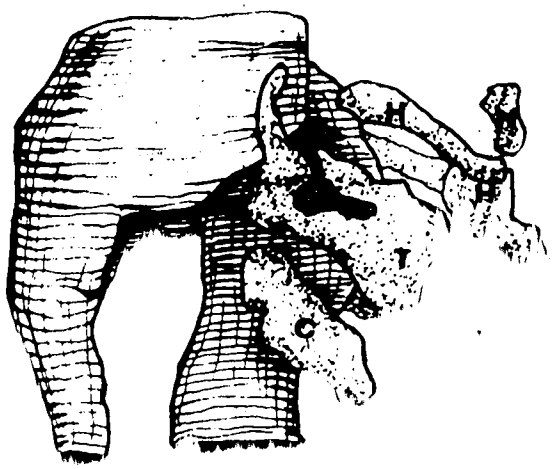
The changes worthy of mention observed in this [illegible] are the commencement of post [illegible] union and the [illegible]

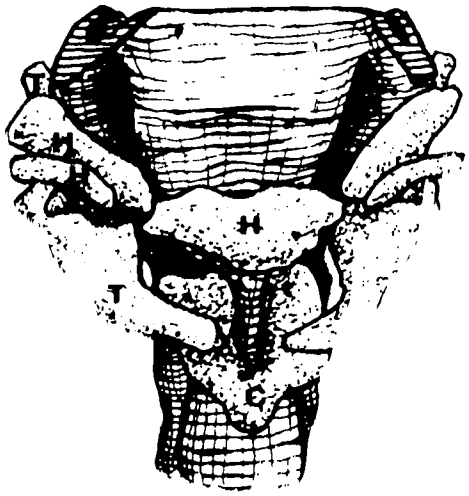
ILLUSTRATION 2

SH81 23.5mm. CR. x40

CARTILAGE







G. SH101 28.5mm.

As expected, the size of the laryngeal skeleton had greatly increased over that seen in SH81. However the most apparent change was the threefold increase in the size of the cricoid anlage.

The thyroid anlage had doubled in size but the circumference of the thyroid foramina was proportionately smaller. This reduction in the circumference of the foramen indicated the onset of its occlusion. The concavity of the laminae was slightly greater than in the previous reconstruction and the laminae had almost met in the midline at their anterior inferior margins. The superior horns are proportionately smaller than in the SH81 reconstruction but the inferior horns had grown to significant prominence. The lower border of the inferior horns were adjacent to the midpoint in cricoid height (see Illustration 1).

The posterior growth of the cricoid anlage had made considerable progress over that of the previous reconstruction and posterior fusion was well under way, although incomplete (Plates 22A, 22B, and 23A). This partial posterior fusion left midline gaps that were clearly evident in the reconstructions. As a result of this posterior growth and fusion, the posterior border of the cricoid was now beyond the posterior border of the thyroid as seen in the adult. This permitted the development of the cricoid

The arytenoid anlages presented themselves as crescent shaped masses located anterior and lateral to the superior margin of the cricoid anlage (Plate 21A). Only the muscular processes of the arytenoid primordia were evident. The apical and vocal processes had not reached the pre-chondral stage of chondrification.

H SU105 40.0mm.

Approximately one week later in development the cricoid and thyroid anlages had doubled in size while the arytenoid primordia had tripled in size. The overall appearance of the laryngeal skeleton at this stage was late pre-chondral and adult like in morphology with the exception of the vocal processes of the arytenoid primordia that had yet to undergo chondrification.

The thyroid laminae were partially fused anteriorly by the midline (Plate 21B) and the thyroid foramina remained patent but otherwise the morphology of the thyroid was adult like. The superior and inferior horns as well as the oblique lines were adult in form. The lower border of the inferior horns were situated adjacent to the midpoint of the cricoid height as is in the adult.

The posterior fusion of the cricoid anlage was complete although the development was not as mature (Plate 22A and B). The superior tubercles that form in part the articulation for the arytenoid anlages had developed

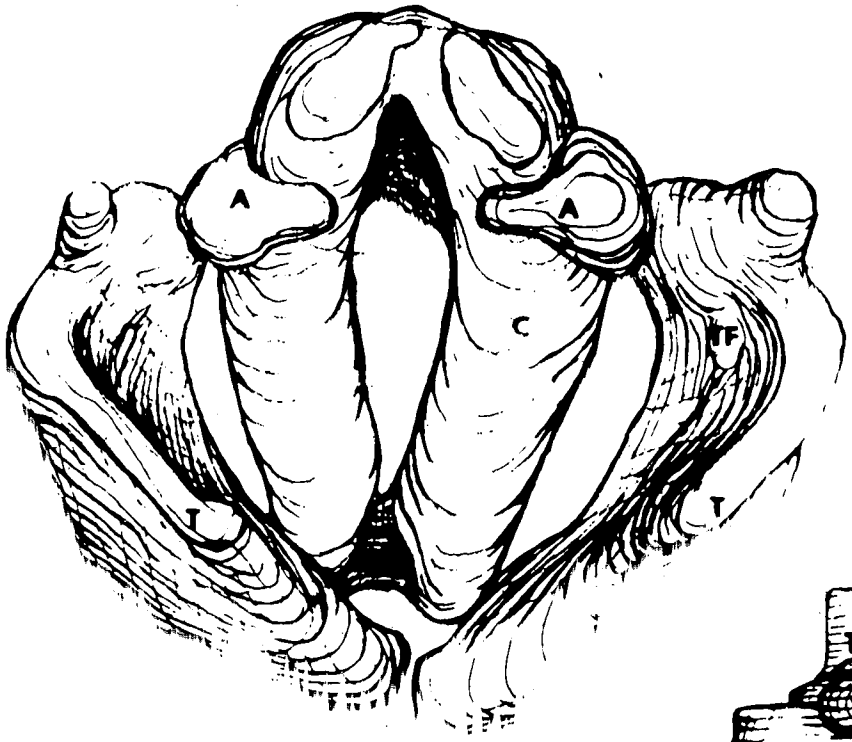
the superior margin of the cricoid lay in the same horizontal plane as the thyroid foramina.

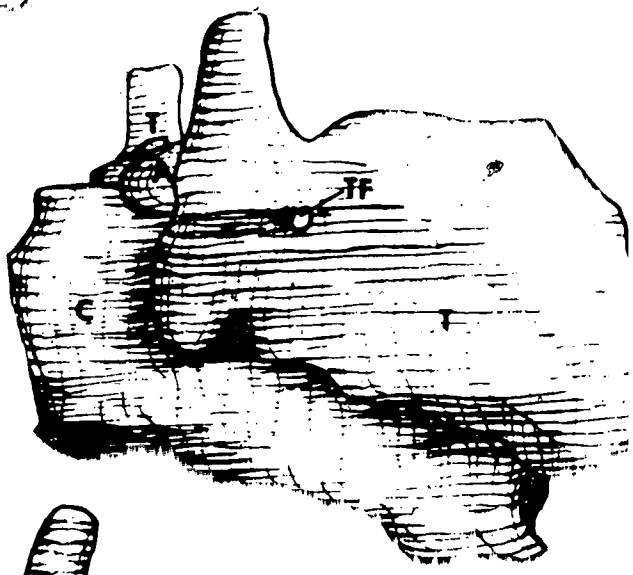
The muscular processes of the arytenoid primordia had developed to an adult like condition but the rest of the primordia was underdeveloped (Plate 25B). The apical processes had begun chondrification but, as one can see from the illustrations, the process is not complete (Plate 24B and 25A). The vocal processes were still in the dense mesenchyme stage of development (Plate 26A). However the [illegible]

ILLUSTRATION 3

SU101 28.5mm. CR. x75

CARTILAGE





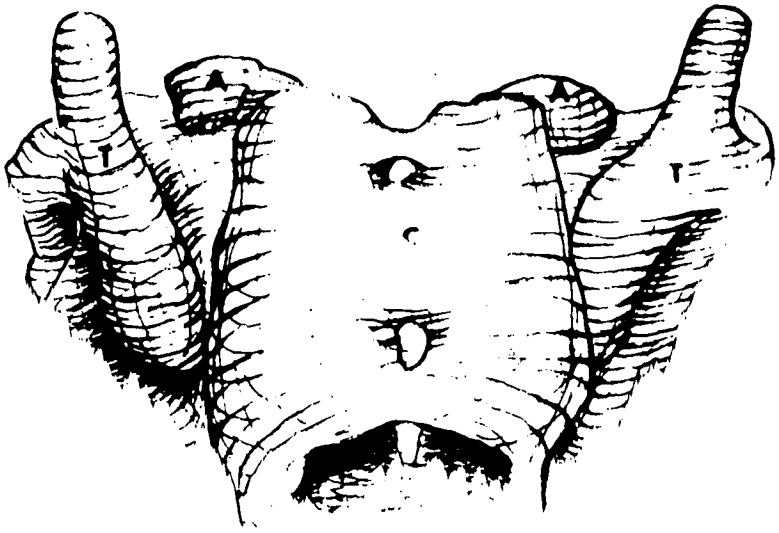
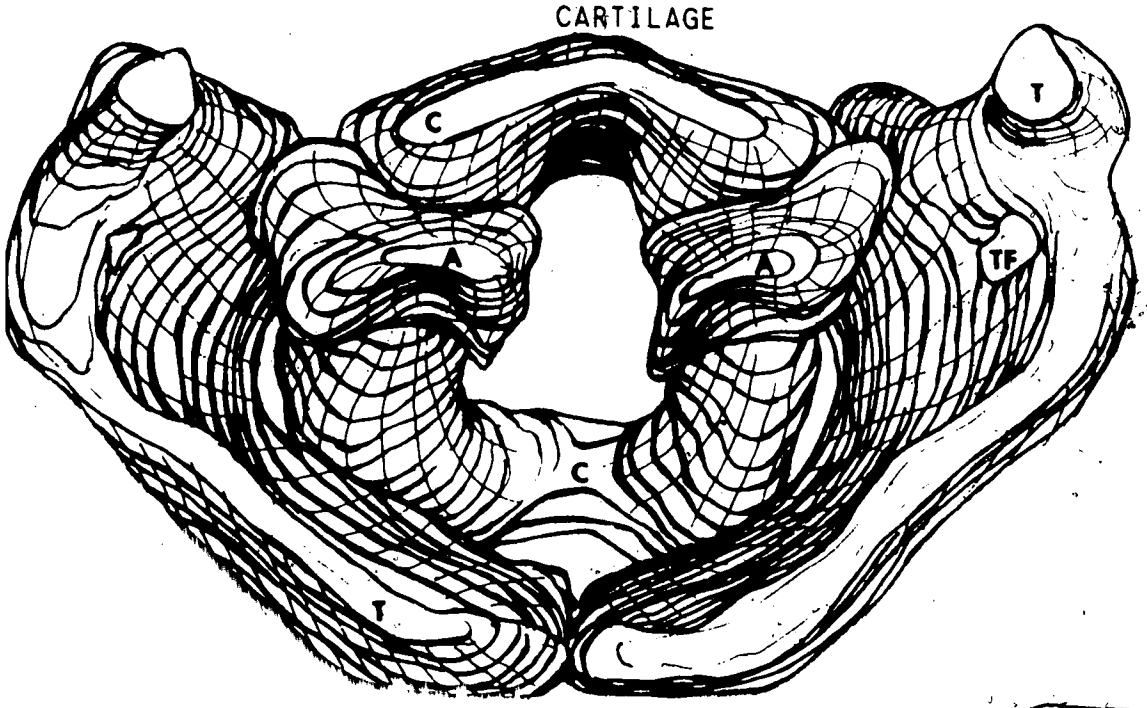


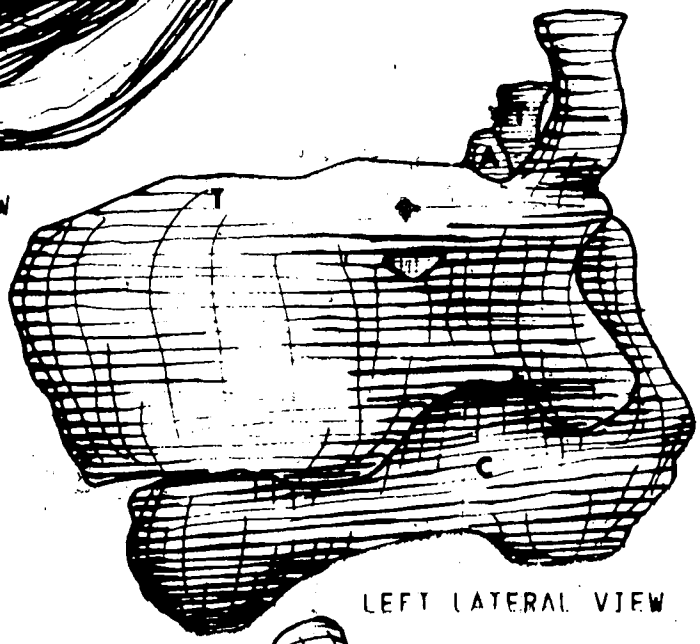
ILLUSTRATION 4

SH105 40.0mm.CR.x75

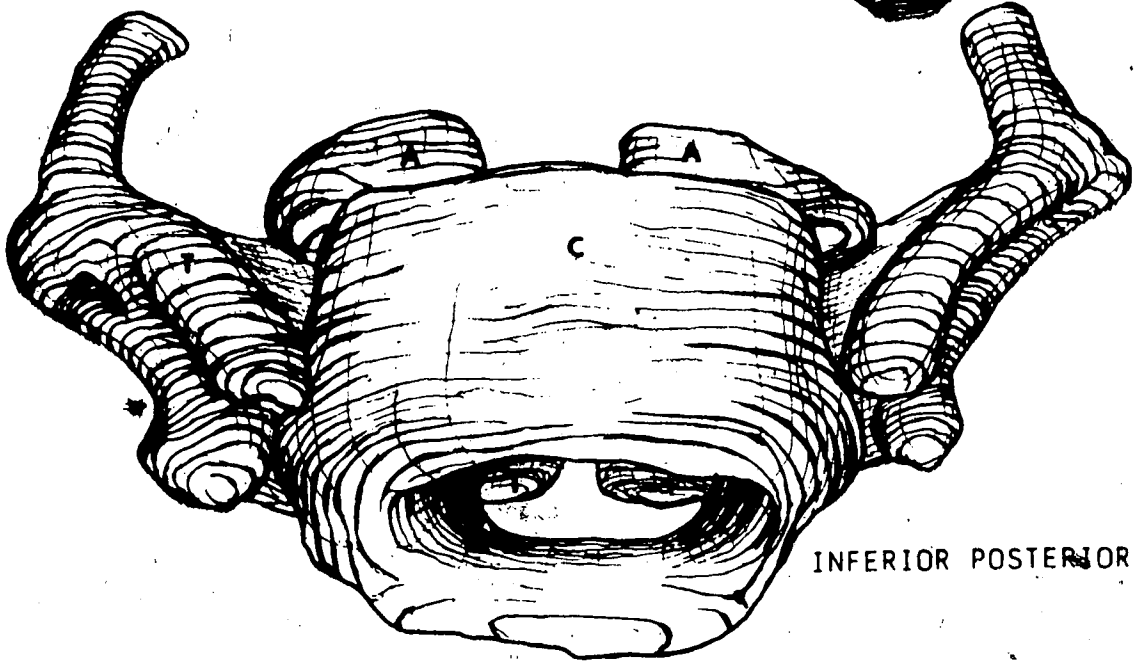
CARTILAGE



SUPERIOR VIEW



LEFT LATERAL VIEW



INFERIOR POSTERIOR VIEW

I. SH106 71.0mm.

The reconstruction of the laryngeal skeleton of this fetus had doubled in size over the previous reconstruction and was very adult-like in appearance. Chondrocytes were recognizable denoting that the chondral stage of development had been reached. The characteristics that were not adult-like consisted of a persisting right thyroid foramen and underdeveloped synovial joints at the various articulation sites.

The laminae had totally fused in the anterior midline forming a single thyroid anlage (Plates 29, 30 and 31). The thyroid foramen had occluded on the left and all other characteristics of this cartilage were adult-like.

It was interesting to note that the hyoid was adult like in form but not in position. The hyoid overhung the thyroid at this stage and must therefore "ascend" to attain its adult position. More will be said about this topic in the discussion.

The cricoid primordium was adult-like in both form and position (Plates 32 and 33). This aspect will be reviewed further in the discussion. Further development of the synovial joints and growth were the only requirements needed to complete its development.

The arytenoid anlages were adult like in shape and position (Plates 29, 30, and 31). Synovial joint formation was noted (Plates 32 and 33) and therefore only growth and joint completion were required to bring about its adult

condition. It should now be apparent that growth, occlusion of the thyroid foramen, and formation of the various joint capsules are the remaining stages necessary in the development of the laryngeal skeleton.

J. SH108 110.0mm.

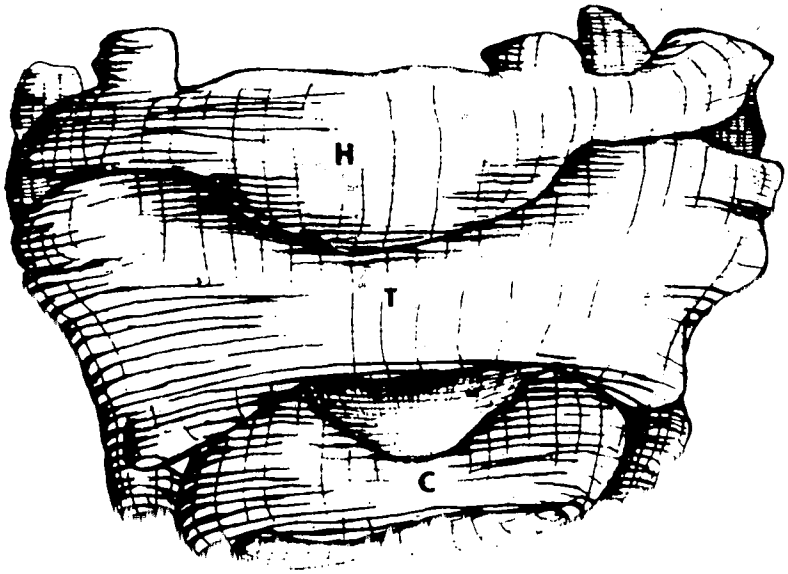
The laryngeal skeleton of this fetus did not show any advanced morphological characteristics, with the exception of joint development, over the previous reconstruction (Plates 34 and 35). The synovial joint cavities were apparent but the thickness of tissue section in both SH106 and SH108 prevented detailed observation of the development of these diarthrodial joints and therefore further discussion is not warranted.

The outcome of this reconstruction was not satisfactory. Numerous sections were either lost or misplaced within the series and, as stated in the method, any incompleteness in the serial sections will skew the reconstruction as was the case here. The major fault apparent in this reconstruction was the superior-inferior compression owing to missing sections. The result was an underdeveloped thyroid height and lack of robustness of the muscular processes of the arytenoid primordia. Despite the inadequacies the model as a whole was quite useful in that it revealed little developmental advance over the SH106 reconstruction. Therefore, the laryngeal skeleton was adult like in shape prior to 110.0mm. This specimen was more

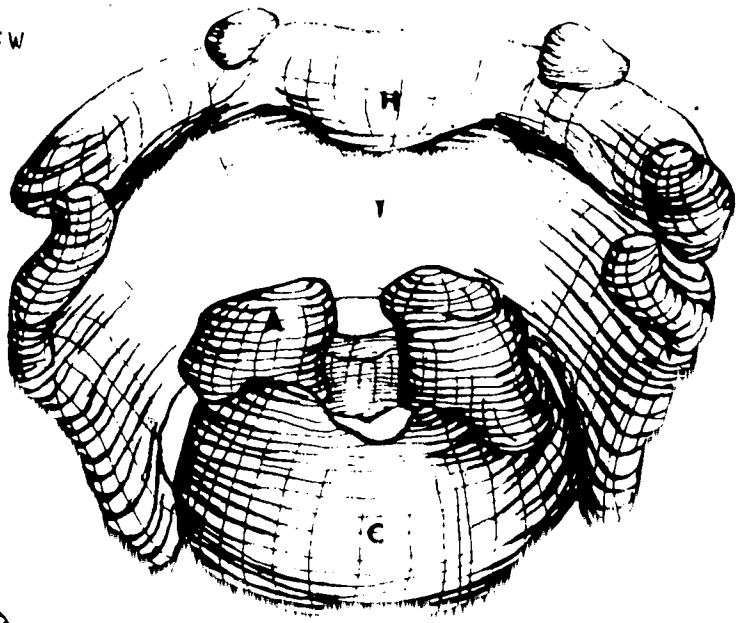
useful for the following investigations of muscle development.

SN106 71.0mm. CR. x25

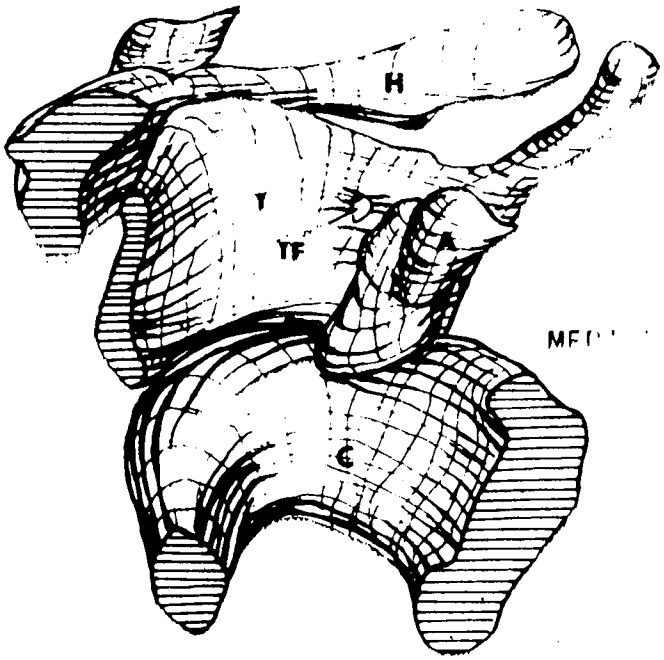
CARTILAGE



ANTERIOR VIEW



POSTERIOR SUPERIOR VIEW



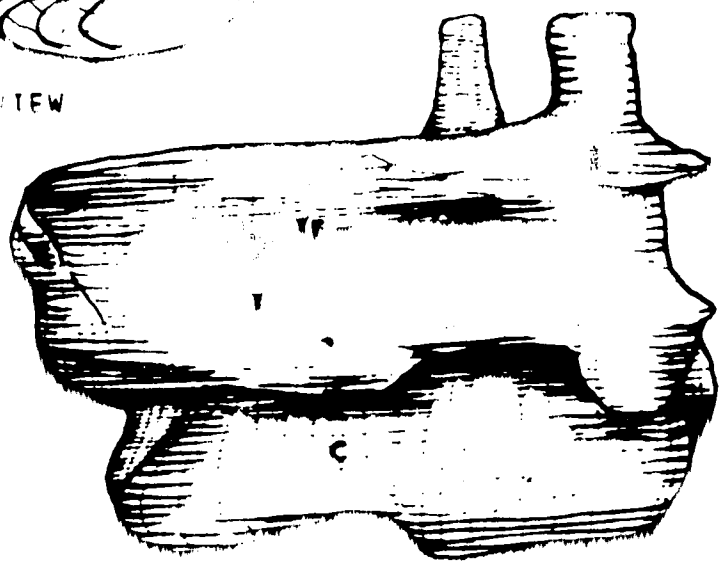
MEDIAL [illegible] OF SCAPULA

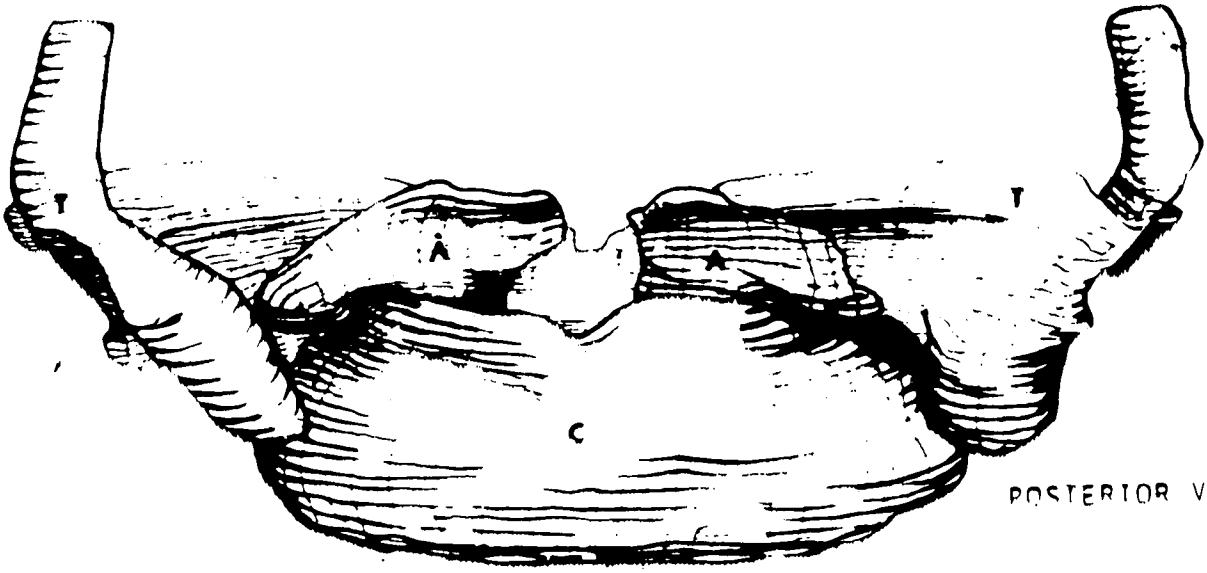
ILLUSTRATION 6

SH108 110.0mm..CR.x30

CARTILAGE

SUPERIOR VIEW





POSTERIOR VIEW

V. Development of the Intrinsic Laryngeal Musculature

As previously mentioned in the literature review some authors (Frazer, 1910 and Hast, 1970) contended that the intrinsic laryngeal musculature arose from two circular mesodermal concentrations (reminiscent of that seen in fishes). The following observations that pertain to this topic were interpretations from photographs of the laryngopharynx prior to the differentiation of the intrinsic laryngeal mucles.

The earliest sign of a mesodermal concentration adjacent to the laryngopharynx was noted in a 4.75mm embryo (Plate 1). This concentration became increasingly dense and then separated into two concentrations (SH28, Plate 2). The inner concentration increased its density until at one point the muscle anlages could be identified. This was the case in the 11mm (SH45, Plate 3) specimen where the interarytenoid, posterior cricoarytenoid, and the thyroarytenoid primordia were in the premyoblastic stage of development. By 12.5mm (SH43) the previously mentioned primordia remained in the same stage but now the cricothyroid anlage could also be placed in this grouping.

At no time in the study could the inner condensation be observed differentiating into a constrictor layer similar to that of the outer constrictor. The term constrictor implies function and therefore I will refer to these masses as "condensations". Nonetheless this condensation does give rise to most of the intrinsic laryngeal musculature, the

exception being the cricothyroid. More will be mentioned on this topic in the discussion.

A SH80 17.5mm and SH83 18.0mm.

The earliest observable sign of the myoblastic stage of muscle development appeared in these specimens. The inner mesodermal condensation was a bilateral column paralleling the laryngeal lumen from the level of the internal laryngeal to the inferior laryngeal nerves. These two columns were bridged superiorly by the interarytenoid primordium (Plate 5B). The outer mesodermal condensation was the continuation of the inferior pharyngeal constrictor (Plate 6B) that develops into the cricothyroid muscle. The arytenoid primordia had obtained myoblastic status at 17.5mm (SH80) whereas the posterior cricoarytenoid primordia were in a transition from the premyoblast to the myoblast stage. The primordia seen in SH83 were similar to those in the last slightly more distinct.

B SH51 20mm.

As previously mentioned, this specimen although catalogued as 15.5mm was, by comparison with other specimens, probably closer to 20mm and was considered as such in this study. The reconstruction of this specimen revealed the partial separation of the cricothyroid anlage from that of the inner muscular mass. This was a timely important in that it re-established contiguity and not continuity

The transversely oriented fibers of the cricothyroid (Plates 9D and 10A) were continuous with the inferior pharyngeal constrictor and were attached to both the cricoid and thyroid anlages.

At this stage the inner conglomeration consisted of an undifferentiated mass (although individual primordia were discernable) of all the remaining intrinsic muscles of the larynx. The myoblastic interarytenoid anlage was continuous inferiorly with the myoblastic posterior cricoarytenoid primordia (Plate 9B) which was continuous inferiorly and anteriorly with the thyroarytenoid mass (Plate 10A and 10B). Both the thyroarytenoid and cricothyroid primordia were in transition from the [illegible] stage of development.

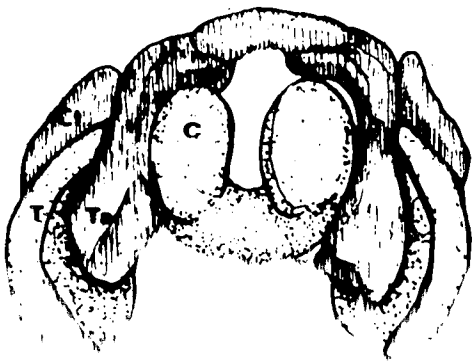
[illegible] SU94 32.5mm

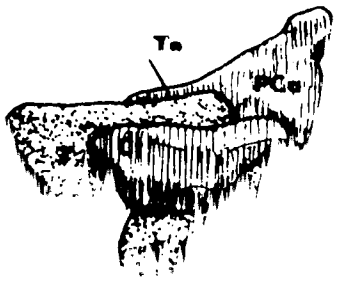
The musculature of SU94 was somewhat advanced over that of SU57 but was very similar to SU81. The interarytenoid anlage (Plates 11A and 11B) was distinctly myoblastic and for the first time was seen attached to the arytenoid primordia. Both the thyroarytenoid and cricothyroid masses had obtained an early myoblastic status. Because of the close morphological similarities to SU81 this region will not be discussed further. [illegible]

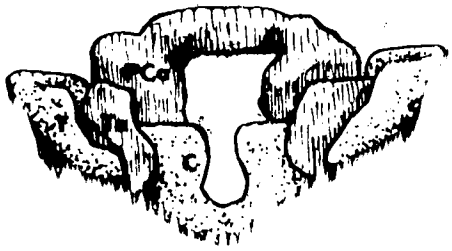
ILLUSTRATION 7

SHE[illegible] 20 0mm. / R. x75

CARTILAGE AND MUSCLE







D SH81 23.5mm.

By this stage the interarytenoid muscles had differentiated out from the inner conglomeration (Plate 15) as a distinct and separate entity. The rapid growth of the cricoid and thyroid cartilages had initiated the separation of the posterior cricoarytenoid primordia from the thyroarytenoid primordia (Plate 15B). There was no evidence of the differentiation of the lateral cricoarytenoid primordia from the thyroarytenoid mass. All of the intrinsic muscles at this stage of development

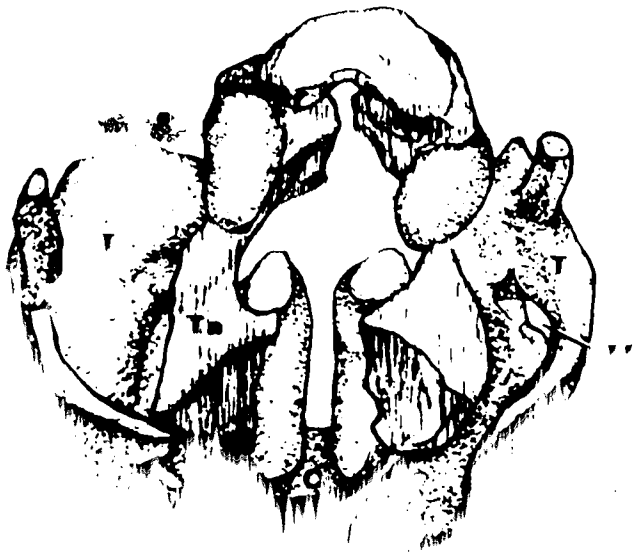
F SH91 26.0mm

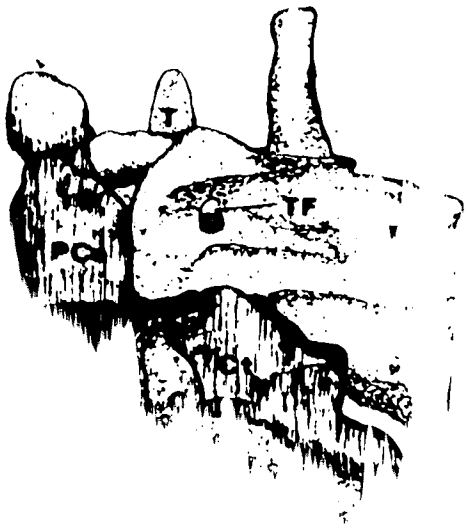
All of the intrinsic musculature seen in this specimen was classified in the late myoblastic stage of development. The posterior cricoarytenoid primordia were still in close apposition with the thyroarytenoid mass (Plate 15A). The lateral cricoarytenoid primordia had not differentiated from the thyroarytenoid mass. The cricothyroid primordia had begun differentiation from the thyroarytenoid mass. This

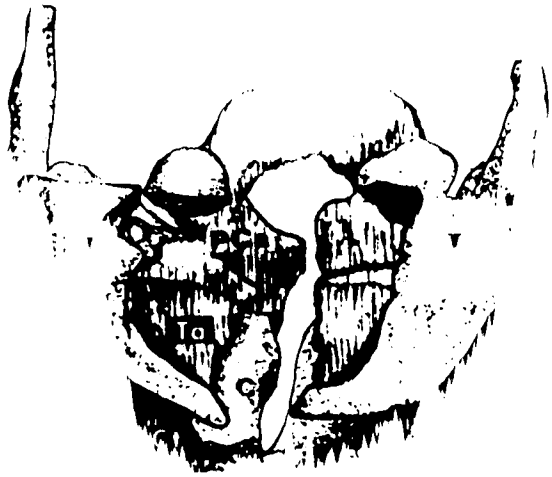
ILLUSTRATION 8

SHEEP 23.5mm CR ×40

CARTILAGE AND MUSCLE







F SU101 28 5mm.

The muscles of this specimen were again classified as late myoblastic. The transverse interarytenoid primordium was quite distinct (Plates 20A, 20B and 21A) but the oblique fibers were not discernable at this stage. The separation of the posterior cricoarytenoid thyroarytenoid mass was almost complete (Plates 21B, 22A, and 22B). The apparent connection seen in Plates 22A and 22B, is for the most part, the inferior laryngeal nerve. The cricothyroid anlages had undergone further separation from the inferior pharyngeal constrictor (Plates 21A, 22B and 23A). There was evidence as yet for the [illegible]

[illegible] SU105 40.0mm

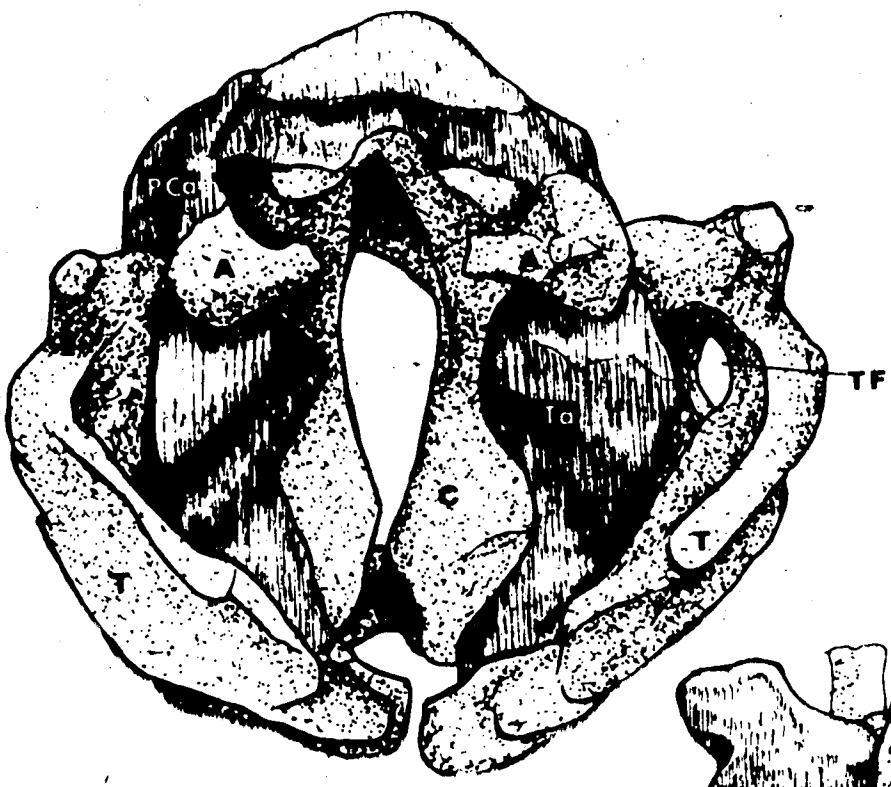
Approximately one week later in development muscle fibers were distinguishable (my1 last stage) and most of the muscles were adult like in their gross morphology. The transverse arytenoid muscle was well developed and the initial appearance of oblique fibers can be observed in Plates 24A and 4B. The posterior cricoarytenoid primordia were adult like in shape and position and for the most part distinct from the other intrinsic muscles (Plates 26A, 26B, 29A). The lateral cricoarytenoid primordia had initiated their division from the thyroarytenoid mass (Plates 5B, [illegible] and 29). The thyroarytenoid muscle (Plate 26B) and the [illegible] were adult like in position

and shape. The cricothyroid muscle and the inferior pharyngeal constrictor were distinguishable as seperate

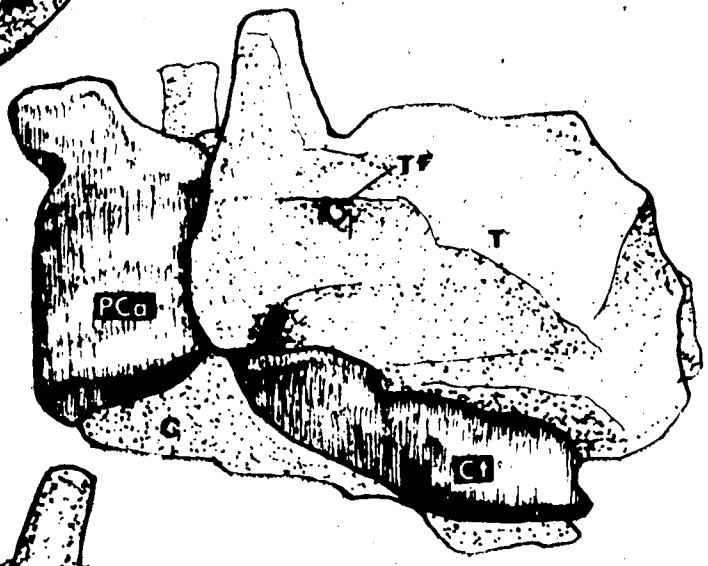
ILLUSTRATION 9

SH101 28.5mm.CR.x75

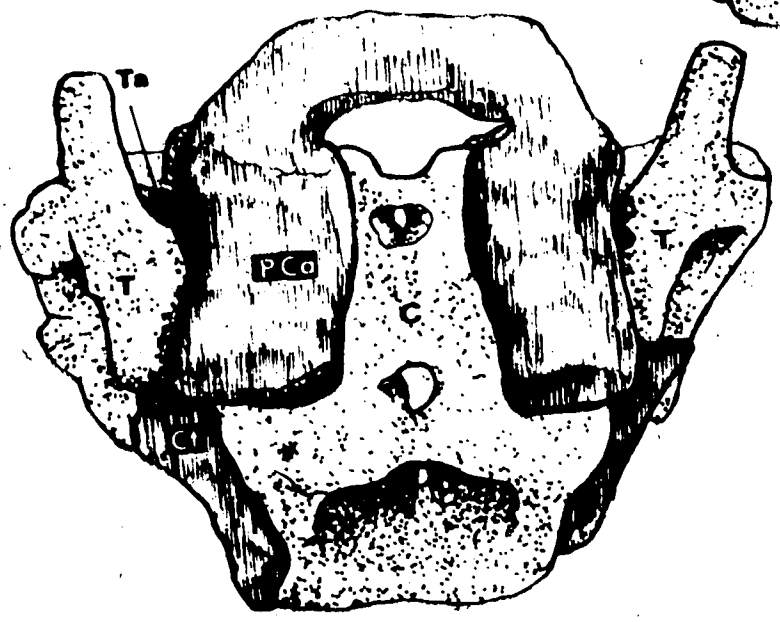
CARTILAGE AND MUSCLE



SUPERIOR VIEW

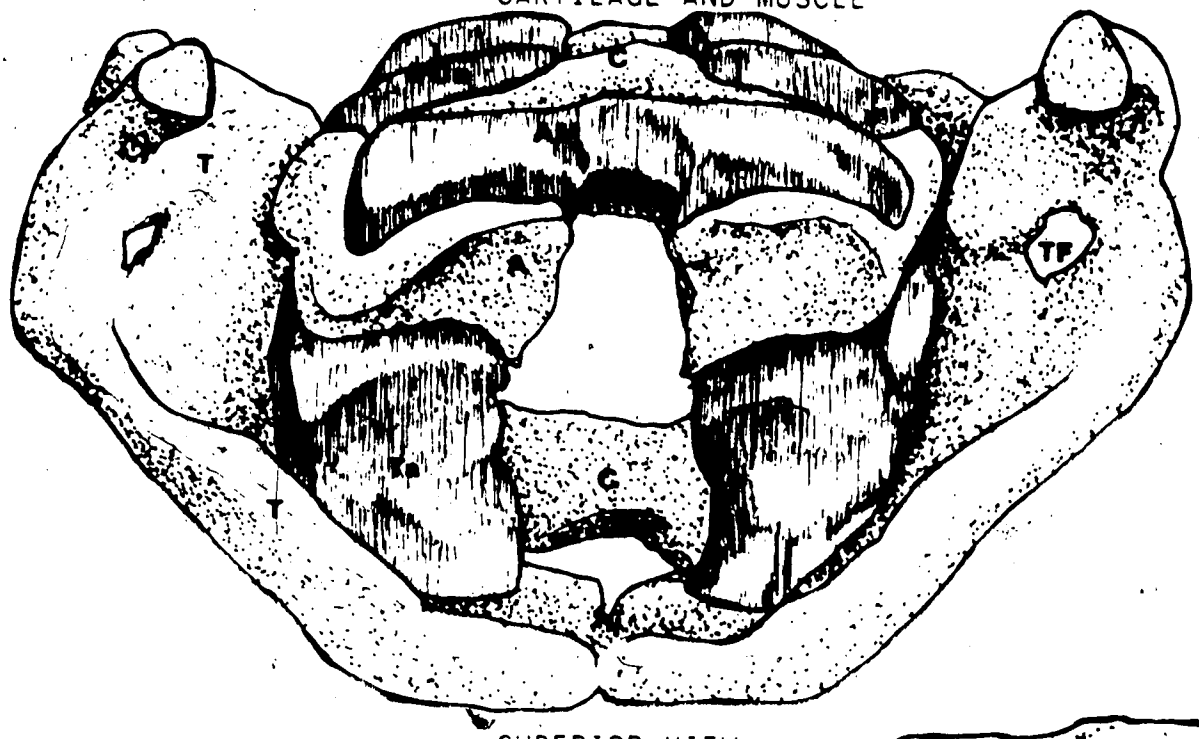


RIGHT LATERAL VIEW

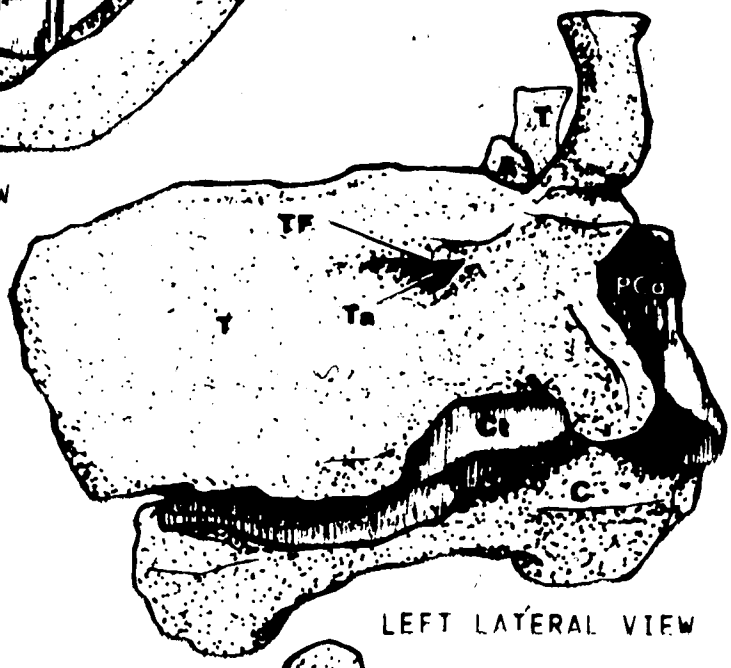


POSTERIOR VIEW

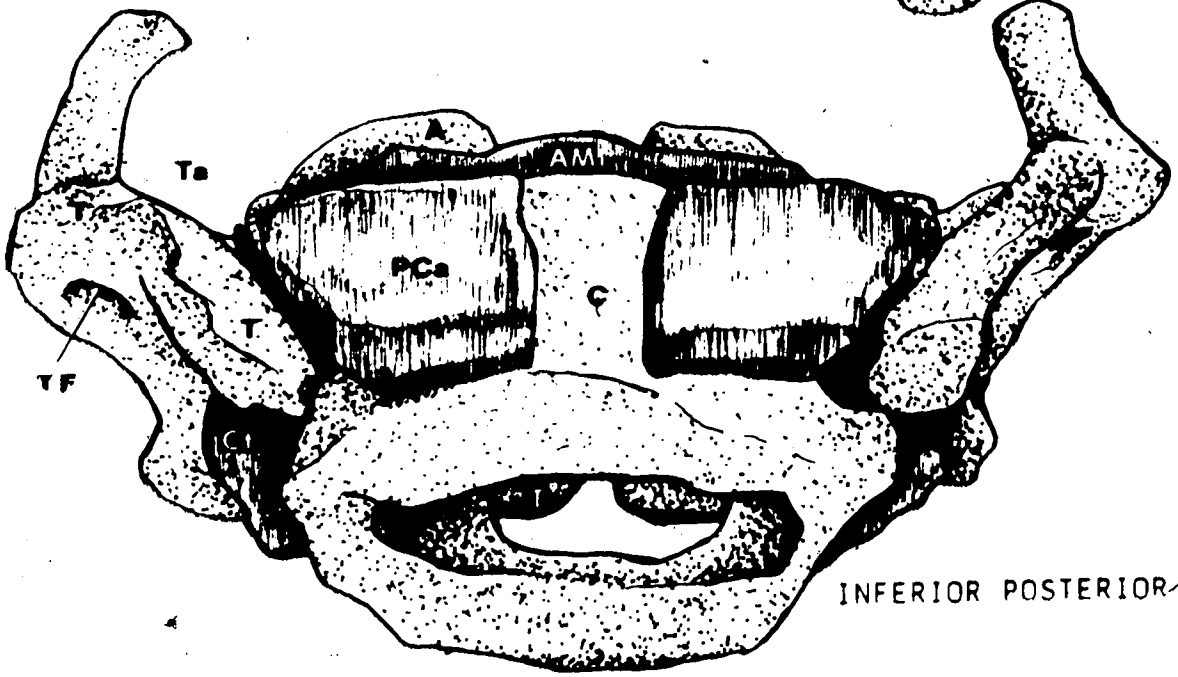
ILLUSTRATION 10
SH105 40.0mm. CR. x75
CARTILAGE AND MUSCLE



SUPERIOR VIEW



LEFT LATERAL VIEW



INFERIOR POSTERIOR VIEW

H. SH106 71.0mm.

The muscle fibers of this 71.0mm. specimen were, with few exceptions, quite distinct and adult-like and should be considered in the late myotube stage of myogenesis. The transverse arytenoid muscle was well developed but required growth to attain the adult condition (Plates 30A, 30B, and 31A). The oblique arytenoid fibers were beginning to differentiate from the transverse fibers but were not as yet distinct (Plate 30B). The thyroarytenoid (Plate 31A), posterior cricoarytenoid and the cricothyroid (Plate 32B) muscles appeared adult-like in shape and position. The lateral cricoarytenoid (Plates 32A and 32B) was basically adult-like in shape and position. The vocalis primordia had began their differentiation from the thyroarytenoid (Plate 31B).

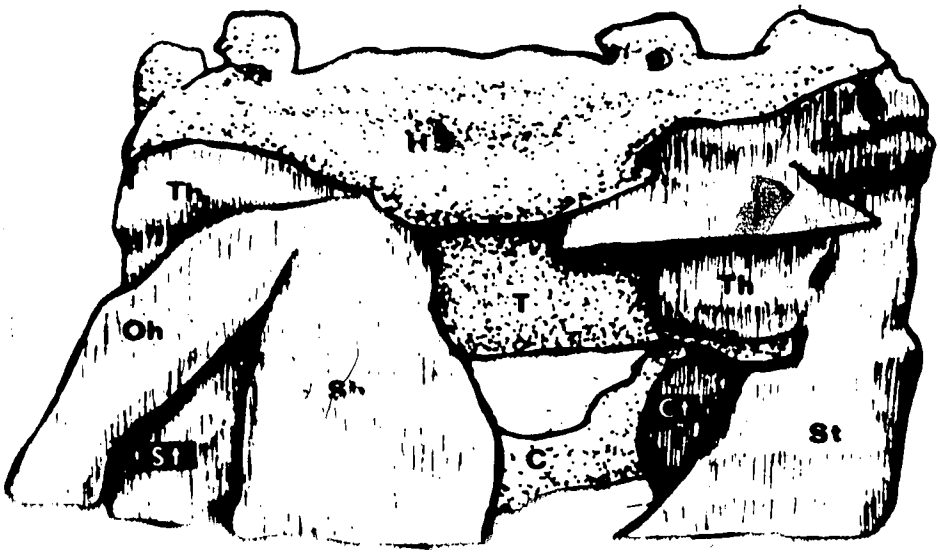
I. SH108 110.0mm.

The muscle fibers were definitely in the late myotube stage and gross morphology of this specimen was more distinct than that of SH106. Some notable changes however were apparent over the SH106 specimen. The continuation of the aryepiglottic muscles was traced to that of the oblique arytenoid fibers (Plate 34A and 34B). The cricothyroid muscles had assumed a more anterior position and the vocalis muscle (Plate 35B) was distinct. The remaining intrinsic musculature had not changed appreciably enough to warrant further discussion.

ILLUSTRATION 11

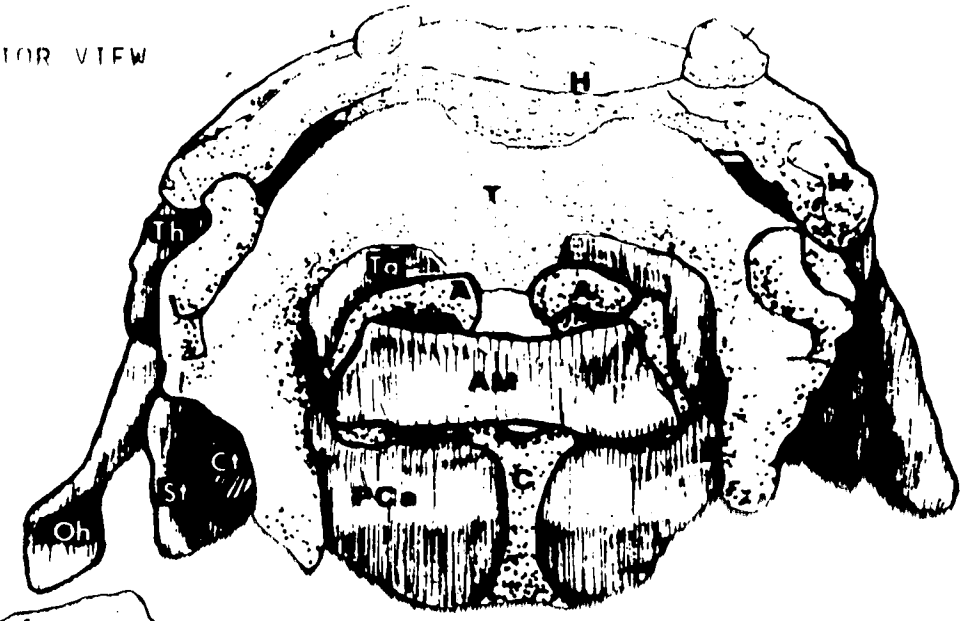
SH[redacted] 71.0mm.CR.x25

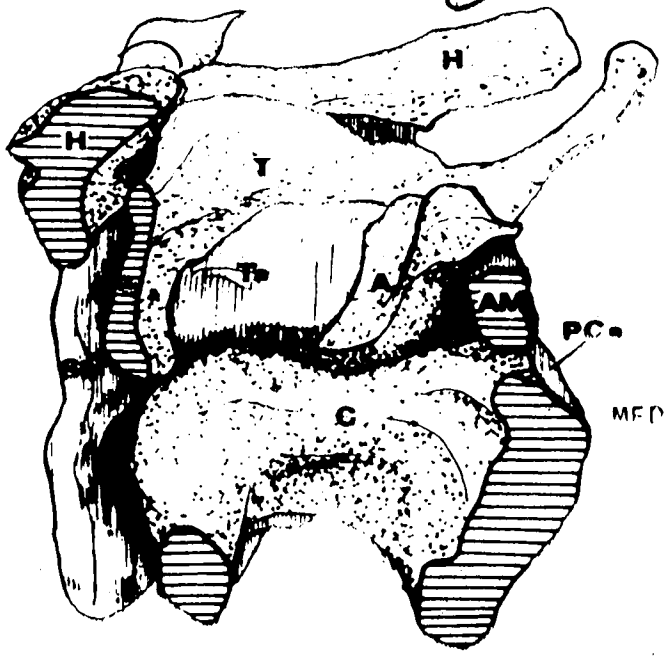
CARTILAGE AND MUSCLE



ANTERIOR VIEW

POSTERIOR SUPERIOR VIEW



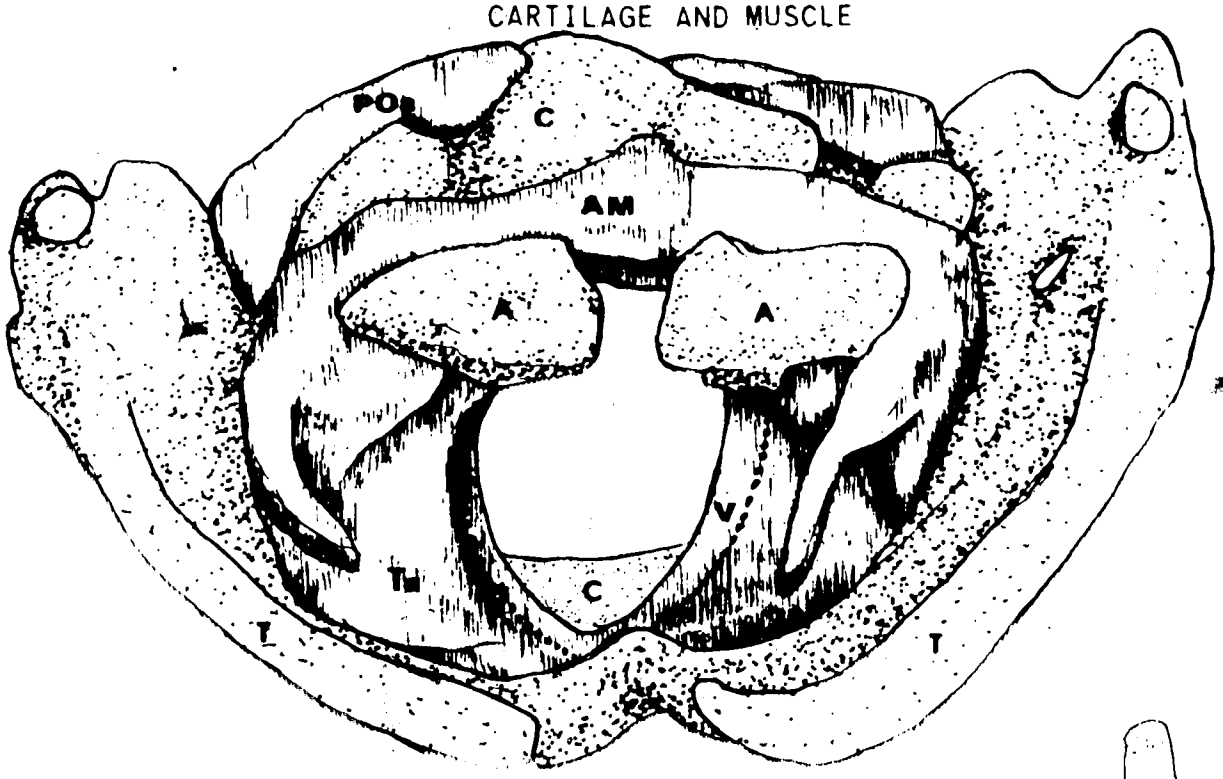


MEDIAL VIEW OF PARTIALLY SECTIONED MODEL

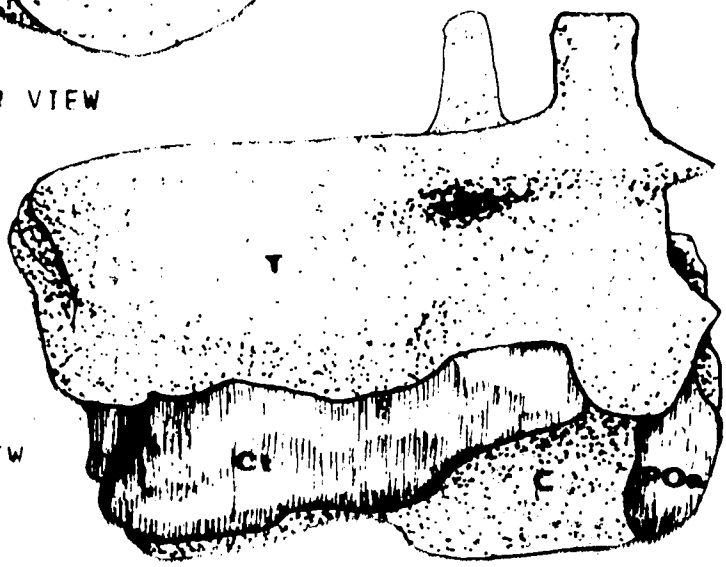
ILLUSTRATION 12

SH108 110.0mm.CR.x30

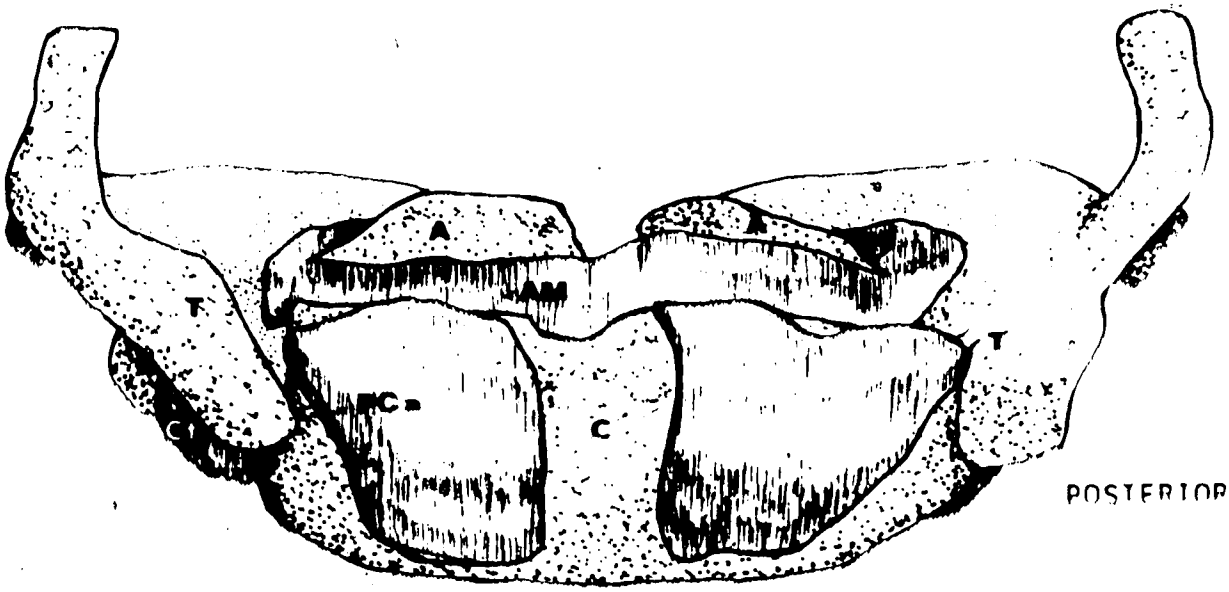
CARTILAGE AND MUSCLE



SUPERIOR VIEW



LEFT LATERAL VIEW



POSTERIOR VIEW

VI. Summary of Laryngeal Development

The following is a concise summary of information from both the literature review and from my study. All of my findings will be denoted with an asterisk.

A single pharyngeal mesodermal condensation surrounded the laryngopharynx at 4.75mm. (*). Shortly thereafter the single pharyngeal condensation differentiated into an inner and an outer condensation that became more distinct as time proceeded (5.0mm., Frazer, 1910; 6.6mm., Frazer, 1910; 7.0mm., (*) & Frazer, 1910; 8.5mm., (*) & Frazer, 1910)

The interarytenoid primordium was discerned at 9.0mm. (Hast, 1970). The posterior cricoarytenoid primordia (Hast, 1970; Lisser, 1911) and the thyroarytenoid primordia (Lisser, 1911) were discernable at 10.5mm. The premyoblastic state of the interarytenoid, posterior cricoarytenoid and the thyroarytenoid were discerned by me at 11.0mm.(*) The premyoblastic cricothyroid primordia(*) and the dense mesenchymal stage of the four segments of the thyroid anlage ((*) & Lisser, 1911) were discernable at 12.5mm. The presence of the cricothyroid and thyroarytenoid primordia were confirmed at 13.0mm. (Hast, 1970).

The ventral arcus of the cricoid anlage was dense mesenchyme at 14.3mm.(*). At 16.0mm. an apparent continuity of the mesenchymal cricoid and arytenoid anlages were noted (Frazer, 1910).

The four segments of the thyroid anlage and the ventral arcus of the cricoid anlage were precartilage at 17.5 and

18.0mm.(*). At the same time, the interarytenoid primordium was myoblastic, the posterior cricoarytenoid primordia were in a transition between the premyoblastic and myoblastic stage and all other muscle primordia were premyoblastic. The U-shaped cricoid anlage and the four segments of the thyroid were prochondral whereas the arytenoid primordia were precartilage at 20.0mm.(*), The thyroarytenoid and cricothyroid primordia were in a transition from the premyoblastic to the myoblastic state of myogenesis at 20.0mm.(*). The superior horns of the thyroid anlage were beginning to fuse with the laminae at 22.0mm (Frazer, 1910).

At 22.5mm. the arytenoid primordia achieved a prochondral status and both the thyroarytenoid and the cricothyroid primordia were myoblastic (*). The inferior horns of the thyroid were discernable as downgrowths from the superior horns and superior horn laminae fusion were noted at 23.5mm.(*). Posterior chondrification of the cricoid anlage was beginning at 26.0mm (*). At the same time, all the muscle primordia were distinct but separation from the inner muscle mass had not begun (*)

Between 27 and 32mm. the thyroid laminae were about to meet ventrally and the thyroid foramina were beginning to occlude (*) The musclular processes of the arytenoid primordia were discernable and the cricoid was relatively complete (Muller et al., 1981). The vocalis muscle was, for the first time, discerned (Muller et al., 1981).

At 35.0mm. the cricoid primordium was relatively complete and the vocal processes of the arytenoid primordia were mesenchyme (Frazer, 1910).

By 40.0mm. posterior chondrification of the cricoid was complete and the thyroid laminae were partly fused in the anterior midline (*). The apical processes of the arytenoid primordia were distinct and the vocal processes of the arytenoid primordia were precartilage (*). The oblique arytenoid and the vocalis muscles were discernable and the remaining muscles began to separate (*). Initial development of the synovial joints were noticed at 40.0mm. (*).

The skeleton was chondral and the muscles were at the myotube stage of myogenesis at 71.0mm (*) The anterior midline fusion of the thyroid was complete and both the cricoid and the arytenoid primordia were adult-like (*) Early formation of the synovial joint cavities were discernable at 71.0mm (*)

The oblique arytenoid muscles were continuous with the aryepiglottic muscles and the vocalis primordia were distinct by 110.0mm (*) The synovial joint cavities seemed further developed but the thickness of tissue sections prevented detailed analysis of the process of joint formation.

VII. Discussion

A. Discrepancies of Specimens in the Collection

As previously mentioned in the results, SH51 had the appearance of being a later developmental stage than was catalogued. This specimen represents a later developmental stage than both SH80 (17.5mm.) and SH89 (18.0mm.) but was not as advanced as SH94 (22.5mm.). The cataloging error noted here, again, makes me stress the caution that must be incorporated into an interpretation of the developmental stage of an embryo based solely upon its size. This error can most likely be attributed to an exaggerated cervical or lumbar flexure in the embryo. This exaggeration would skew the CR measurement taken prior to sectioning. To avoid such confusion in the future, staging the embryos of the B. F. Skinner collection in a manner following that developed by Theiler (1972) would be useful (see below).

Another area of inaccuracy noted in this study relates to the incompleteness of the SH100 sectioning. Not only was it incomplete but the ordering of the sections was also in many places incorrect. Because a number of sections were missing from this series, the reconstuction was compressed in a superior inferior direction. This compression skewed the conception of the reconstruction. Nonetheless the overall morphology gained by the reconstruction demonstrated little developmental advance over that of SH100. This warranted its use because it demonstrated that the

developmental process was relatively complete at 71.0mm.

B. Staging Systems

Staging of human embryos was developed by Mall and later expanded by Streeter into developmental "Horizons" (O'Rahilly, 1979). Recently, this system has been revised by O'Rahilly and was renamed the Carnegie Staging System. The system of staging bases its foundations on the morphological state of a developing embryo and not upon size or age. Age is not an accurate means of describing the developmental state of an embryo because developmental states of individuals from the same age class could be either advanced or retarded (O'Rahilly, 1979). Size is also not an accurate means of describing developmental state because the degree of cervical and lumbar flexion varies from specimen to specimen and can therefore skew the crown-rump measurement. Unfortunately, this system covers only the embryonic period (1-8 post-ovulatory weeks) and is limited application to my study.

C. Cartilage Development

Cricoid

One area of controversy in the literature concerning the laryngeal skeleton was the development of the cricoid. Frazer (1910) contended that the cricoid undertakes a bilateral mode of development (development from two sides with a later midline fusion), whereas Kallius (1911) and

Lisser (1911) reported a unilateral mode of development (development from a single mass that is present on both sides of the midline). Both the photography and reconstructions of this study definitely supported a unilateral mode of development. At no time in the cricoid's development was there evidence to support a bilateral mode of development as Frazer puts forth. Even in the early mesenchymal stage of development the most condensed portion of the cricoid was the ventral arcus. Because the cricoid is fused ventrally only at the inferior margin initially and mesenchymal throughout it remaining extent, a section taken at midlevel could perhaps give the appearance of a bilateral mode of development. Interpretations from sections without reconstruction could be misleading as indeed this could have been the case with Frazer (1910). He reconstructed a 16mm. and a 2mm. embryo but the critical period of cricoid development is absent from this study [illegible] time somewhere between these embryos.

Further support for a unilateral mode of development comes from clinical cases of laryngeal atresias (Smith and Pain, 1965). If the cricoid was found to be patent ventrally in the case of laryngeal atresia then this might suggest mode of development from both sides of the midline that later fuse ventrally. In their classification of laryngeal atresias the cricoid was always fused ventrally and open dorsally. This atresia was purely suggestive of an [illegible] that would prevent [illegible]

posterior chondrification and was supportive of the unilateral developmental process.

Lymphatic drainage of the deep structures of the larynx (muscles and cartilage) also support the mode of cartilage development proposed here. Deep lymphatic drainage below the vocal folds does communicate across the midline (Johner, 1970). This is also evident in by carcinoma metastases from one side of the larynx to the contralateral cervical nodes (Johner, 1970). This supports the unilateral (cricoid) development below the true folds. Deep lymphatic drainage above the vocal folds does not communicate across the midline. This supports the bilateral development of the aryteroid cartilages above the vocal folds.

Thyroid Foramen

The closure of the thyroid foramina occurred somewhat arbitrarily and indeed in some cases did not close completely in the adult (Testut, personal communication). Such cases of persistent thyroid foramina in adult specimens have been observed by me. Closure of the thyroid foramen on the left but not on the right was evident from the U100 (71.0mm reconstruction. This was again apparent in the CU108 (110mm) reconstruction but opposite sides were occluded / patent. Failure of the thyroid foramina to close bilaterally does not seem to produce any clinical problems in the normal adult.

It is interesting to note that in some embryos of approximately 20mm (Plate 14) [illegible] the thyroid

passing through the thyroid foramina. Muller et al. (1981) were able to trace this nerve and maintain that it was a branch of the external laryngeal nerve. Studies of comparative anatomy in the literature provide no insight into the significance of this nerve but its transiency probably indicates that it is a remnant of the phylogenetic history of the species (Negus, 1962; Wind, 1970).

Corniculate and Cuneiform Cartilages

The development of the corniculate and cuneiform cartilages were not observed in this study because the specimens of necessary age were lacking from the collection. According to Hast (1970) the corniculate cartilages began chondrification towards the end of the third month whereas the cuneiform cartilages began their chondrification in the seventh month.

Epiglottic Cartilage

Although the development of the epiglottic cartilage was not part of my study, a brief mention of its development is appropriate. At the end of the embryonic period no cartilage was observed in the epiglottis (Tucker and O'Rahilly, 1972). Fibroelastic cartilage was first observed in the epiglottis at five months but it was not until the eighth month that the definitive appearance was noted (Tucker and Tucker, 1975).

Postnatal Laryngeal Skeletal Growth

Kahane (1978, 1982) studied the postnatal growth of the laryngeal skeleton from the embryonic to adulthood in both male

and female cadavers, measuring both the linear dimensions and weight. With the exception of the anteroposterior dimension of the thyroid cartilage, the intracartilage proportions from prepuberty until adulthood remained relatively stable. Therefore, while the cartilages increased in size, they changed little in shape (Kahane, 1982).

The anteroposterior growth of the thyroid cartilage in the male was three times as great as in the female. The change between prepuberty and adult was 15.04mm. male vs. 4.47mm. female. The absolute increase in all linear dimensions (length, height, and width) of the thyroid and cricoid cartilages were two to three times greater in the male than in the female (Kahane, 1982). The absolute weight increase of the individual cartilages were: thyroid > cricoid > arytenoid as would be expected. Again, the absolute weight increase was two to three times greater in the male vs. adult female laryngeal skeleton (Kahane, 1982).

The Assistance of Muscle Differentiation by Cartilage Growth

A concept worthy of noting here is the separation of muscle masses by the process of cartilage growth. This was evident in two instances in laryngeal development (Hast, 1970). The downward growth of the inferior thyroid cornua separated the outer mesodermal condensation into the cricothyroid muscle and the inferior pharyngeal constrictor posteriorly. In a similar manner, the growth of the arytenoid cartilages separated the interarytenoid muscle from that of

the posterior cricoarytenoid muscles.

D. Muscle Development

The inner constrictor

The debate in the literature regarding formation of most intrinsic musculature of the larynx from an inner "constrictor" (Frazer, 1910 and Hast, 1970, 1972) as opposed to *in situ* development (Lisser, 1911) from the surrounding mesoderm is an interesting problem. Perhaps this problem is simply a question of semantics, as the cricothyroid is derived from the inferior pharyngeal constrictor which, in a semicircular fashion, surrounds the inner mesodermal mass. Frazer (1910), for the sake of simplicity, might have termed the latter the inner constrictor. If so there should be no controversy over this topic. If, on the other hand, he did, indeed, attribute a constrictor status to this inner mass, then this study disagrees with his interpretation. Lisser (1911) noted that the direction of fibers in a frontally sectioned specimen yields little evidence for the constrictor status implied by Frazer (1910) of the inner mass of mesoderm. Nonetheless my study indicated that the intrinsic laryngeal musculature, with the exception of the cricothyroid, differentiate from this inner mesodermal condensation. It must be emphasized that the close relationship of the laryngeal components in the inner mesodermal condensation may be contiguous and not continuous, as proposed in the literature (see below). This

problem cannot be solved with the techniques adopted in this study but perhaps could be solved using cell tracers, such as tritiated thymidine, in experimental animals (Weston, 1970).

The onset of development of the muscles proceeded in a superior to inferior direction and not simultaneously. This concept in itself is interesting because the development of the laryngeal cartilages did not follow this pattern but, in fact, followed a pattern reverse to that of its phylogeny (see below). That is, instead of the arytenoids developing first and the cricoid and thyroid developing simultaneously, the reverse pattern was demonstrated.

It should be stressed at this time that the adult innervation of the intrinsic laryngeal musculature and the inferior pharyngeal constrictor support a bimodal origin (from the inner and outer condensations). A fundamental embryological corollary states that if a nerve supplies more than one muscle, these muscles have the same mesodermal origin (Arey, 1974, p430). The cricothyroid and inferior pharyngeal constrictor are both innervated by the external laryngeal nerve whereas the remaining intrinsic musculature are innervated by the inferior laryngeal nerve.

Interarytenoid Development

The development of the interarytenoid muscle is somewhat controversial. Hast (1970) considered the interarytenoid muscle to be the first discernable muscle (at 9.0mm.). Hast (1972) after further study decided that the

interarytenoid muscle was probably not discernable until slighty later (ie. at approximately 11mm.). This again stresses the problems inherent in observing differentiating cells. I agree that the myoblast stage of development occurs first in the interarytenoid muscle but development thereafter was slower than the other intrinsic musculature. The interarytenoid muscle was the first to differentiate and the first discernable muscle derived from the inner mesodermal concentration.

Lisser (1911) observed the presence of the interarytenoid primordium as early as 12.5mm. Although there was a sign of premyoblastic tissue observed at 11.0mm. (SH45) and 12.5mm. (SH43), in the present study, I did not consider these muscles distinct but rather a discernible undifferentiated portion of the inner mesodermal condensation. The myoblastic interarytenoid anlage was definitely discernable in the present study by 17.5mm. (SH83).

The interarytenoid development following its differentiation was, in comparison to the cricoid and thyroid, slow. It was not until 40.0mm. (SH105) that the transverse fibers were distinct and not until 110.0mm. (SH108) that the oblique fibers can be considered distinct and in continuity with the aryepiglottic muscle fibers.

Development of the Vocalis Muscle

The development of the vocalis muscle has been discussed very little by previous authors in their

discriptions of the intrinsic laryngeal musculature. Because it has been implicated in the role of changing fundamental frequency during phonation, a brief disscussion must be included (Zemlin, 1981, p202). In my observations during the present study the vocalis began to differentiate at about nine weeks (Plates 27A and 27B). By fourteen weeks (Plate 35B) it was readily distinguishable from its parent muscle, the thyroarytenoid, but had yet to develop to its adult condition. Muller et al. (1981) observed what they believe to be the component fibers of the vocalis muscle as early as the eighth week. According to Konig and von Leden (1961) the vocalis muscle did not differentiate fully until the third year of life. If this is the case, the muscle differentiation precedes development of the vocal ligament (see below).

E. Phylogeny of the Larynx

A brief description of the phylogeny of the larynx is presented here because of its relevance to the discussion. The onset of lungs was first recognized in the fossil record in a Silurian placoderm *Bothriolepis* (Denison, 1941). Breathing of air while surfacing above an aquatic medium with a subsequent dive down into the water required some sort of sphincter to prevent both air escape and water entry into the lungs. This sphincter was the primitive larynx, similar to that found in the present day dipnoan *Lepidosiren* (Negus, 1962). Relaxation of this sphincter would passively

dilate the lumen of the laryngopharynx. More advanced forms of lungfish (*Protopterus*) added dilator muscles to this primitive larynx to increase the efficiency of abduction of the glottis. Amphibians such as *Axolotl* added a pair of arytenoid cartilages to this dilator apparatus and in newts we find the first appearance of a cricothyroid cartilage (Negus, 1962). This musculoskeletal system is essentially the same in alligators and birds but the cricoid and the thyroid separated in the mammals (Negus, 1962). The larynx of *Homo* serves not only as a valve but has also been modified to produce complex sounds during the process of speech.

The ontogeny of the larynx does not closely recapitulate its phylogeny. The arytenoid cartilages do not develop before the cricoid and thyroid but rather after these cartilages. The muscles of the inner condensation have been greatly modified beyond the ancestral function of a valve to participate in phonation. The inner and outer pharyngeal condensations develop almost simultaneously. The outer condensation does not preceed the development of the inner condensation, as one would expect from phylogeny.

F. Pharyngeal Arch Derivatives

The reconstructions in my study do not support the classical pattern of visceral arch derivation of the laryngeal skeleton as postulated in standard textbooks of embryology.

The superior horn of the thyroid cartilage in early development was continuous with the greater horn of the hyoid, which is classified as a third arch derivative. The laminae of the thyroid cartilage developed from single chondrification centers on both sides of the midline and at no time were these laminae continuous with the cricoid. I see no reason to classify the thyroid laminae and the cricoid as both fourth and sixth arch derivatives as seen in embryologic textbooks. I postulate that the superior-inferior horn segments are third arch derivatives and that the laminae are fourth arch derivatives.

The cricoid develops from a single chondrification center ventral to the layngeal lumen that chondrifies in a superior and posterior direction and finally completely encircles the laryngopharynx. Again I see no justification for this cartilage to be divided into different arch derivatives, since the cricoid develops from a single chondrification center. I regard the cricoid as a sixth arch derivative.

The arytenoid cartilages are relatively late in developing, when compared to the thyroid and the cricoid. The arytenoid cartilages also develop at the level of the thyroid foramina. The laryngeal saccule develops just below the arytenoid cartilages. As you will recall in SH81 (Plate 14B) a transitory nerve was observed passing through the thyroid foramen. I speculate that the laryngeal saccules are fifth pouch derivatives and that the arytenoid cartilages

are fifth arch derivatives. These structures are relatively late in developing because they belong to the rudimentary fifth arch which has a poor blood supply. The nerve passing through the thyroid foramen could have at one time been the nerve to the fifth arch.

G. Development of the Vocal Folds

The development of the vocal fold was not closely examined in this study but because of its close association to the skeleton and intrinsic musculature of the larynx, a brief account will be mentioned. According to Frazer (1910) the vocal folds were formed from "chordal nodules" that undergo subsequent atrophy leaving the cords attached to both the thyroid junction and the vocal processes of the arytenoid cartilages. Goerttler (1954), cited in Muller et al. (1981), evidently supported this view and further suggested this mode of development of the human glottis to be fundamentally distinct from that of other mammalian species, although the authors did not explain why this process was distinct.

In an unpublished paper Hirano, Kurita, and Nakashima (1981), cited in Kahane (1982), discussed the postnatal development of the vocal ligament. The authors stated that prior to four years of age the vocal ligament cannot be identified as a histological entity. Between the years of four and sixteen the elastic and collagenous components of the vocal ligament increased in density and at the same time

became orientated in the anteroposterior plane (Kahane, 1982).

Postnatal growth of the vocal folds (as measured by the length dimension increase from prepuberty to adulthood) was observed by Kahane (1982). Kahane (1982) found the male vocal fold growth (11.57mm.,63% increase) to be more than two times greater than the female counterpart (4.16mm.,34% increase). This relative change in the male/female vocal fold length had been described as the most influential factor contributing to the fundamental frequency change in voice at puberty.

H. Laryngeal Web

An interesting observation of some clinical relevance was noted in this study (and in the studies of H. Zaw-Tun, personal comm.) with respect to the development of the laryngeal lumen. Here, as in the case of the duodenal lumen development (Arey, 1974, p253), the lumen occludes and later recanalizes by the formation of many small vesicles. The appearance of the laryngeal lumen at 23.5mm. (SH81), 28.5mm. (SH101), and 40.0mm. (SH105), looking through the glottis was that of a spider's web. Therefore it is probable that stagnation of the developmental process at this stage would result in the annomalous laryngeal web. This recanalization of the laryngeal lumen is a possible area for research in the future because the web can develop at locations above, below, or at the level of the glottis (Paparella and

Shumrick, 1980).

I. Development and Congenital Laryngeal Atresia

Congenital laryngeal atresias have been classified on morphological grounds by Smith and Bain (1965) into three types. These types represent cessation of the developmental process at specific periods of gestation. The morphological characteristics of these three types will be discussed as described by these authors and in relation to my observations relative to developmental age.

Type one was said to encompass both supra and infraglottic atresias. The laryngeal vestibule was represented by a shallow cleft and the laryngeal sinuses were absent. The arytenoid cartilages were fused for variable lengths in the midline. The cricoid cartilage appeared roughly conical in shape and was patent posteriorly. Because the atresia was both above and below the glottis, the posterior deficiency in the cricoid must be quite large. Taking the above information into account, it would seem from the observations during my study, that type one represents an arrest in development sometime in the latter part of the seventh week because of its morphological similarity to the SH81 (23.5mm.) reconstruction (Illustration 2).

Type two involves only infraglottic atresia. Both the laryngeal vestibule and sinuses were normally developed and the arytenoids were separate. The cricoid was described to

be dome-shaped owing to the presence of a conical cavity within. Posteriorly the cricoid cartilage was grooved and was patent inferiorly. The dome-shaped cricoid with posterior inferior patencies was characteristic of the SH101 reconstruction. Therefore, type two represents developmental arrest within the early part of the eighth week (refer to illustration 3).

Type three was characterized by an atresia at the level of the glottis. The laryngeal vestibule and sinuses were normally formed but the glottis was occluded by a membrane composed of fibrous connective tissue and muscle. The arytenoid cartilages were joined by a thin cartilaginous bar at the level of the vocal processes. The cricoid cartilage was normally formed, with the exception of a posterior patency at the level of the glottis. Type three must represent a developmental arrest sometime in the latter part of the eighth week (after SH101 yet before SH105) because posterior cricoid fusion was complete at this stage.

Based on the developmental processes of my study the fusion of the arytenoid cartilages in either type one or type three can be explained only by an upset in the induction process. As you will recall (Bronner-Frazer and Cohen, 1980), the endoderm of the laryngopharynx induces the cartilage development of the larynx. Both type one and type three atresisas contain upsets in the laryngopharynx endoderm at the levels of abnormal cartilage development. This would also account for the lack of posterior cricoid

chondrification, because the cricoid is also in contact with the endoderm of the larynx.

The existence of the tracheoesophageal septum has been disproved by Zaw-Tun (1982). Therefore the mode of formation of laryngeal clefts utilizing the tracheoesophageal septum theory as stated in Paparella and Shumrick (1980, p2434-5) must be revised to incorporate the new data. This is another possible area for future research.

J. The "Ascent" of the Hyoid

Another observation made in passing pertains to the relative position of the hyoid to the laryngeal skeleton. In early stages of development (SH51 and SH81) the hyoid was suspended above the thyroid laminae. During later development the hyoid overhangs the thyroid laminae (SH106). Later still (SH108) the hyoid was suspended above the thyroid laminae in a similar fashion to the earlier development. This fluctuation in spinal level of the larynx during development was also noted by Noback (1923). I postulate that early growth of the laryngeal skeleton causes the thyroid laminae to grow up under the hyoid and that later differential growth in the neck region causes the hyoid to "ascend" to its adult position.

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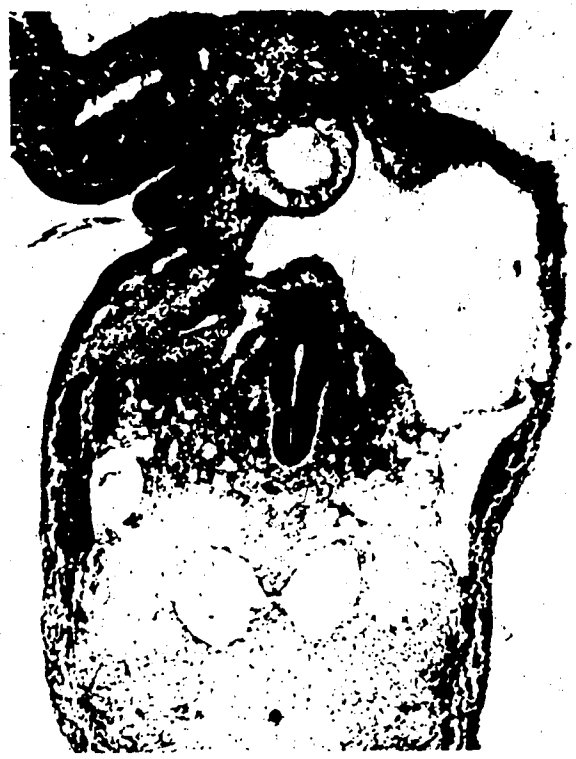
IX. Appendices

Plate 1: SH5 4.75mm.CR.

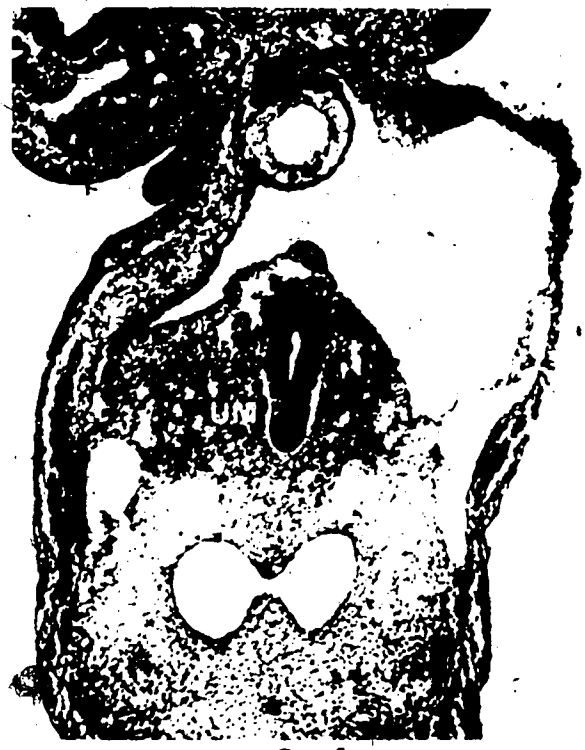
A) 3.2.2: This plate was taken at the upper level of the laryngopharynx. This plate and all of the following plates are placed in the anatomical position so that the top of the page is ventral and the bottom of the page is dorsal. Surrounding the laryngopharynx is a single layer of condensed mesenchyme (the undifferentiated mesenchyme).

B) 3.2.4: This plate represents the successively lower section and again demonstrates undifferentiated mesoderm.

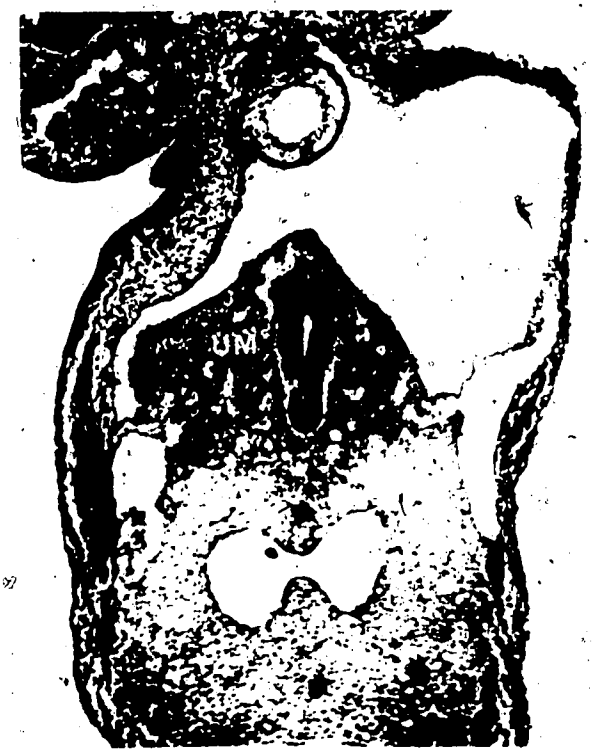
C) 3.2.5: This plate again represents the successively lower section of this embryo. The three photographs demonstrate the continuity of a single column of undifferentiated mesoderm.



3.2.3



3.2.4



3.2.5 x630

Plate 2: SH28 7.0mm.CR.

A) C.5.15: This plate represents a section taken at the upper level of the laryngopharynx. The mesoderm surrounding the laryngopharynx can be differentiated into an inner and an outer mesodermal condensation.

B) C.6.1 and C) C.6.2: These photographs represent successively lower sections of the same embryo and demonstrate the continuity of the inner and outer columns of mesoderm.

SH 28

c.5.15

c.6.1

c.6.2

x630

Plate 3: SH45 11.0mm.CR.

A) F.4.9: This plate represents a section taken at the level of the fourth pharyngeal pouch. Again, both an inner and outer mesodermal condensation surrounds the laryngopharynx.

B) F.4.10 and C) F.4.11: These photographs represent the continuity of the inner and outer mesodermal condensations.

SH45

f.4.9

f.4.10

f.4.11

x630

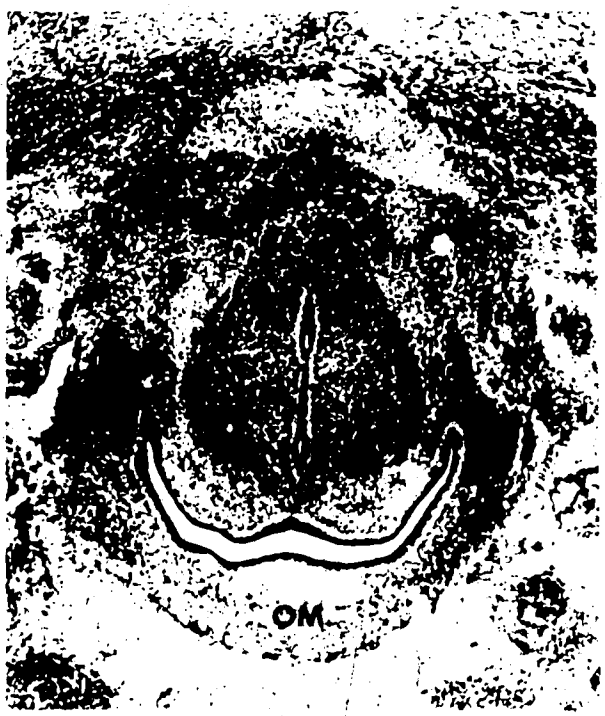
Plate 4: SH11 14.3mm.CR.

A) G.3.6, B) G.3.7, and C) G.3.8: These photographs again demonstrate the inner and outer mesodermal condensations surrounding the laryngopharynx.

SH11

g.3.6

g.3.7



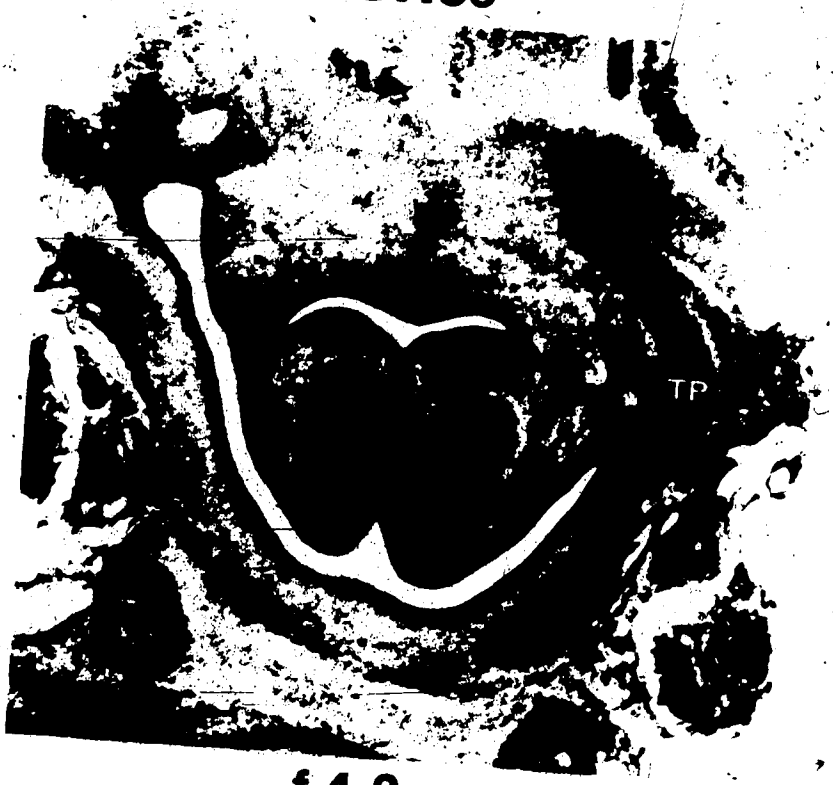
g.3.8 x630

Plate 5: SH80 17.5mm.CR.

A) F.4.2: This photograph represents a section at the uppermost border of the larynx. The superior horn of the thyroid is labeled TP.

B) F.4.10: This plate demonstrates the arytenoid primordia and posterior to these is the interarytenoid primordia.

SH80



f.4.2

f.4.10

x630

Plate 6: SH80 17.5mm.CR.

A) G.1.7: In this photograph the internal laryngeal nerve courses anterior to the thyroid primordium to enter the inner mesodermal condensation. The posterior cricoarytenoid primordia are discernable at the terminating point of the internal laryngeal nerve.

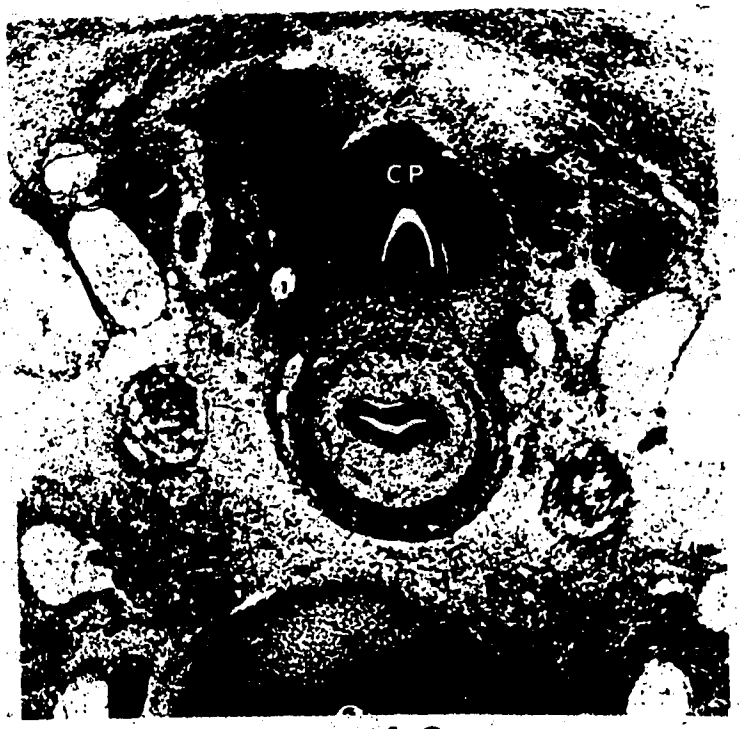
B) G.2.3: The U-shaped outer condensation is discernable surrounding the inner condensation. The posterior cricoarytenoid primordia are in continuity anteriorly with the thyroarytenoid primordia. The nervous tissue amongst these primordia is the inferior laryngeal nerve.

C) G.4.3: This photograph displays the early condensation of the ventral arcus of the cricoid.

SH80

g.1.7

g.2.3



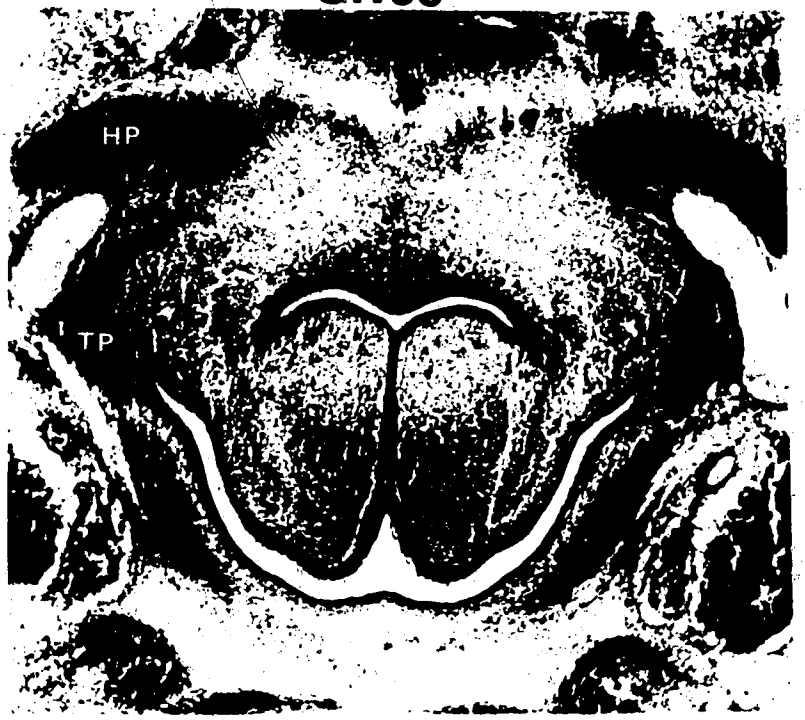
g.4.3

x630

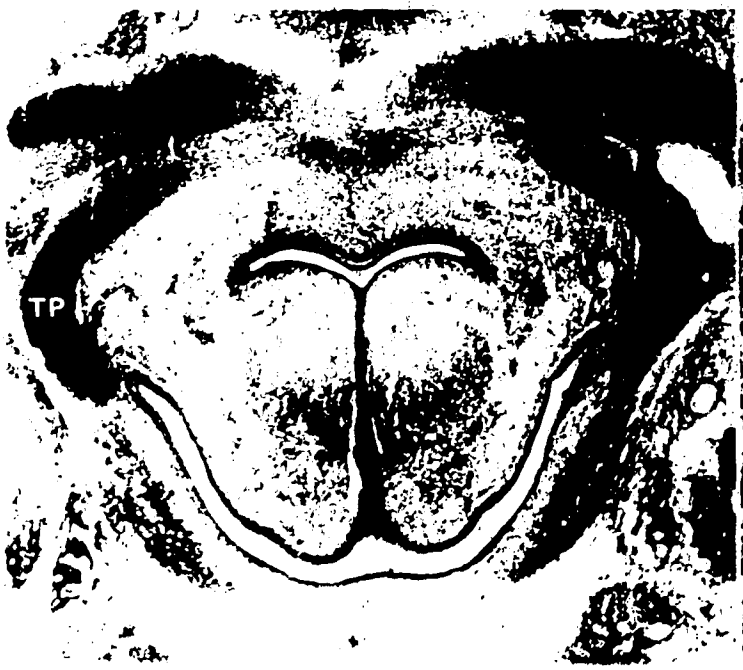
Plate 7: SH83 18.0mm.CR

A) F.2.2, B) F.2.4, and C) F.2.8: These photographs are similar to SH80 but are somewhat more advanced and require no more discussion.

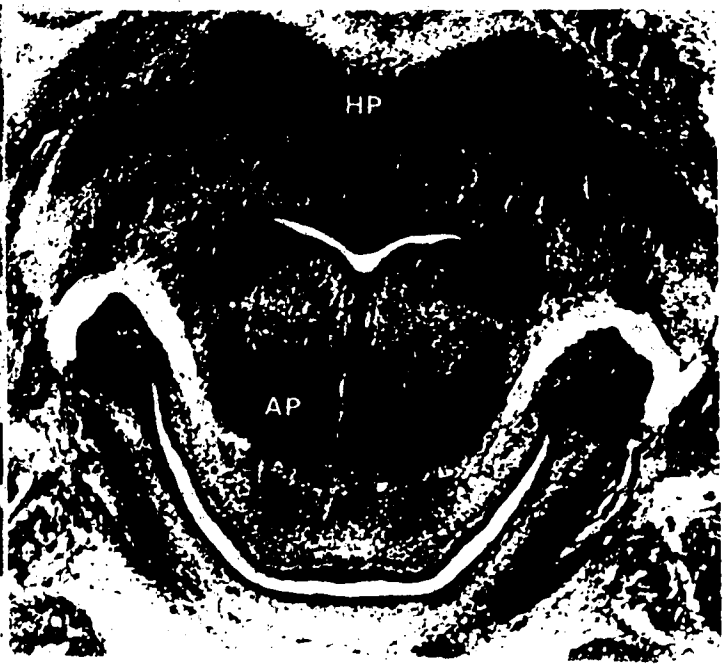
SH83



f.2.2



f.2.4



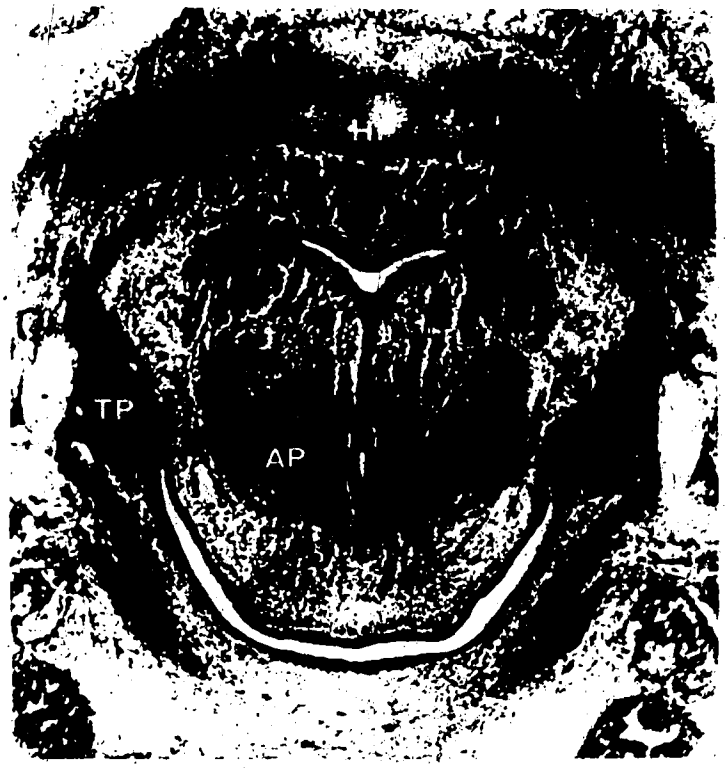
f.2.8

x830

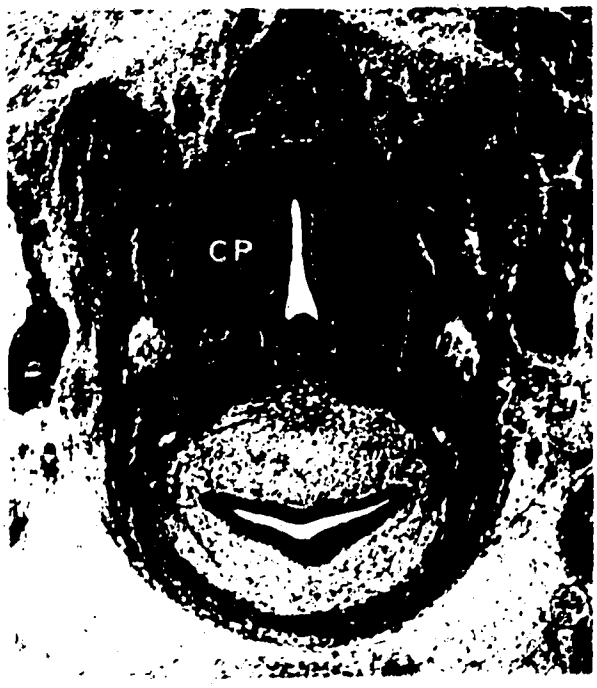
Plate 8: SH83 18.0mm.CR.

A) F.3.2, B) F.4.8, and C) G.1.6: These photographs are again very similar to SH80 except that the cricoid condensation is progressing superiorly.

SH83



f.3.2



f.4.8

g.1.6 x630

Plate 9: SH51 20.0mm.CR.

A) H.3.2, B) H.3.6 and C) H.4.3: These photographs represent the superior horns, the arytenoid primordia, and the transverse arytenoid muscle.

D) H.5.3: Both the posterior cricoarytenoid and the inferior pharyngeal constrictor are present in this photograph. Note how the inferior pharyngeal constrictor loops around the esophagus to attach to the thyroid laminae.

SH51

h.3.2

h.3.6

h.4.3

h.5.3

×630

Plate 10: SH51 20.0mm.CR.

A) I.1.2: The posterior cricoarytenoid primordia are continuous anteriorly with the thyroarytenoid primordia. The inferior pharyngeal constrictor is again attached to the thyroid laminae.

B) I.1.6 and C) I.2.1: The cricothyroid primordia are easily discernable medial to the inferior pharyngeal constrictor. The cricoid and thyroid are prochondral.

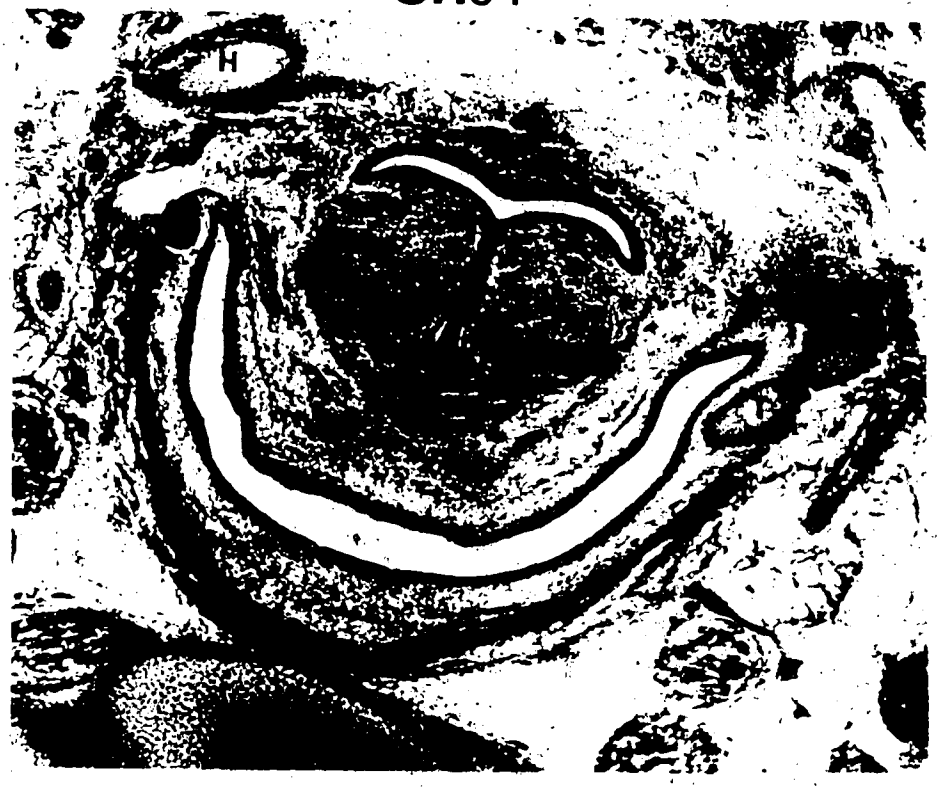
D) I.3.1: The ventral arcus of the cricoid is prochondral.

Plate 11: SH94 22.5mm.CR.

A) M.2.2: Both the superior horns of the thyroid primordia and the transverse arytenoid anlage are easily discernable.

B) M.3.2: The arytenoid primordia are connected by the transverse arytenoid primordium.

SH94



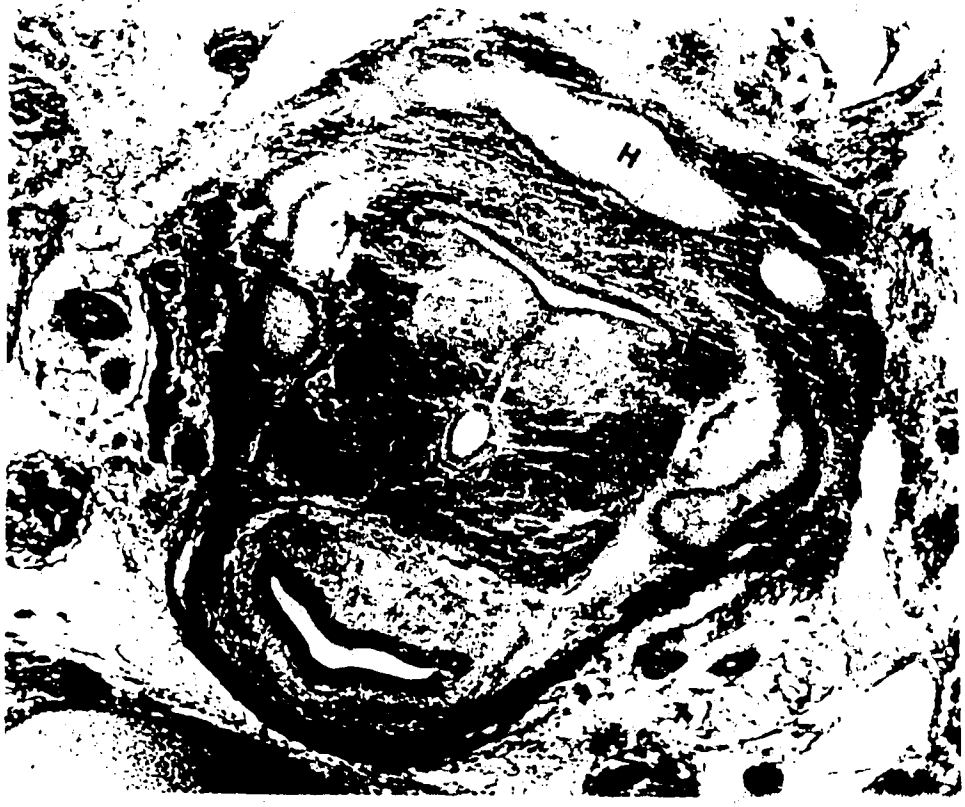
m.2.2

m.3.2 x630

Plate 12: SH94 22.5mm.CR.

A) M.4.4 and B) M.5.3: These photographs display the continuity between the posterior cricoarytenoid and the thyroarytenoid primordia. The continuity is interrupted in (B) by the inferior laryngeal nerve.

SH94



m.4.4

m.5.3 x630

Plate 13: SH94 22.5mm.CR.

A) N.1.2, B) N.3.2, and C) N.4.3: This series of photographs demonstrates the continuity of the cricoid laminae and ventral arcus.

SH94

n.1.2

n.3.2

n.4.3

x630

Plate 14: SH81 23.5mm.CR.

A) P.2.4: This photographic plate demonstrates the myoblastic status of the transverse arytenoid primordium.

B) P.3.5: The prochondral thyroid and arytenoid cartilages along with the myoblastic posterior cricoarytenoid are demonstrated here. The transient nerve that passes through the thyroid foramen is present on the right.

SH81

p.2.4

p.3.5

x630

Plate 15: SH81 23.5mm.CR.

A) Q.1.3 and B) Q.2.1: These photographs demonstrate the continuity of the myoblastic posterior cricoarytenoid and thyroarytenoid primordia.

SH81

q.2.1

q.1.3 x63(

Plate 16: SH81 23.5mm.CR.

A) Q.2.7: This photograph demonstrates the continuity of the myoblastic inferior pharyngeal constrictor and the myoblastic cricothyroid primordia. The contiguity of the myoblastic thyroarytenoid and cricothyroid primordia is also displayed.

B) Q.3.7: This plate demonstrates the initial posterior chondrification of the cricoid anlage.

q.3.7

q.2.7

x630

Plate 17: SH81 23.5mm.CR.

A) R.1.7 and B) R.2.2: These photographs represent sections at the lower border of the prochondral cricoid demonstrating the ventral continuity.

SH81

r.1.7

r.2.2

x630

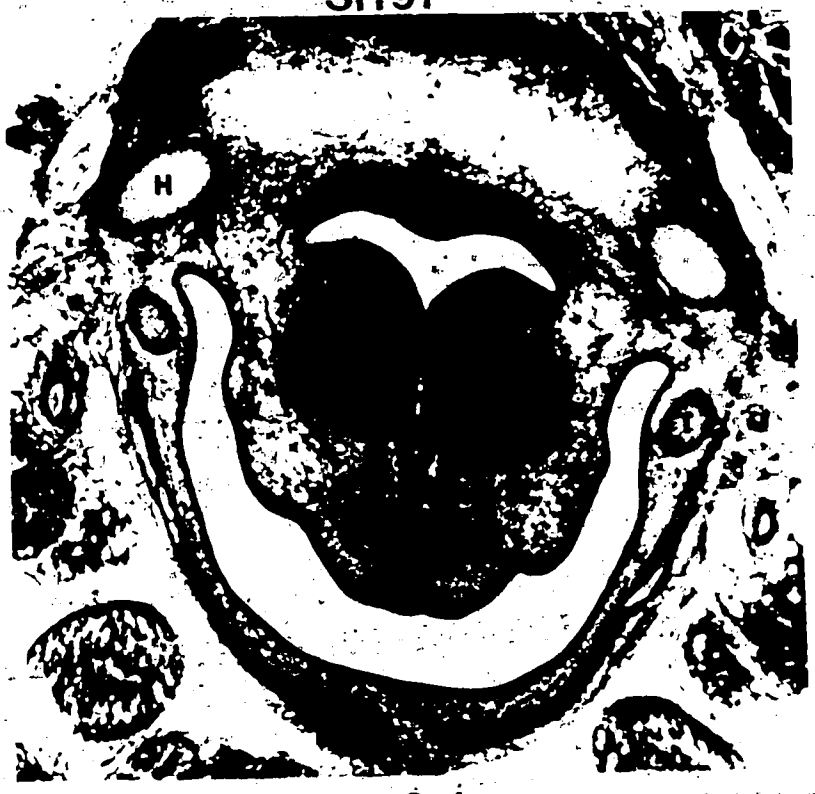
Plate 18: SH91 26.0mm.CR.

A) M.2.1: This plate shows the dense mesenchymal state of the arytenoid apical processes.

B) M.3.7: The myoblastic posterior cricoarytenoid and the thyroid and arytenoid cartilages are represented in this photograph.

C) N.1.8: Posterior cricoid fusion and the continuity between the thyroarytenoid and posterior cricoarytenoid are demonstrated in this photograph.

SH91



m.2.1

m.3.7

n.1.8

×630

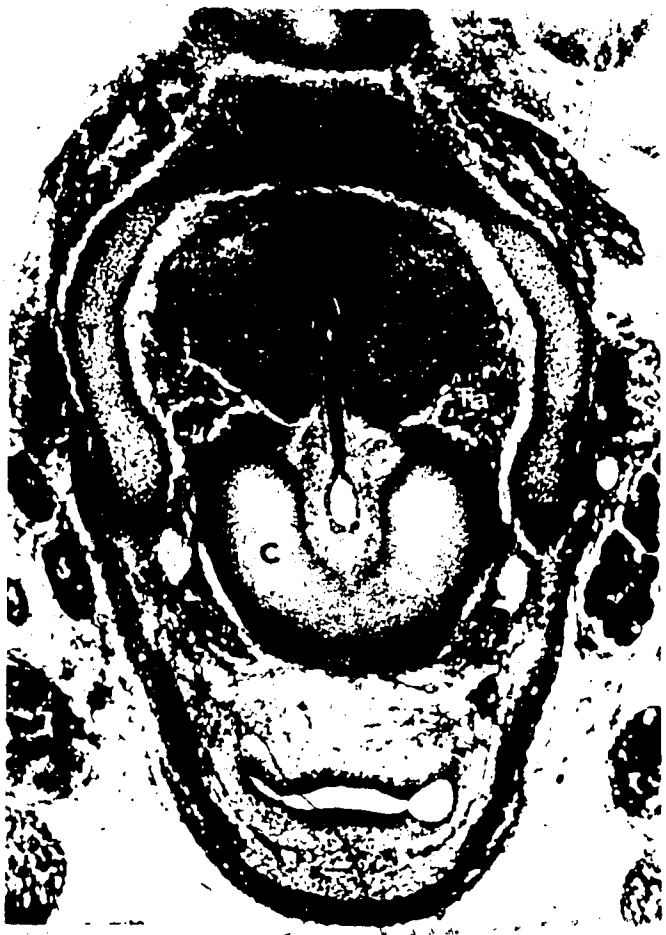
Plate 19: SH91 26.0mm.CR.

A) N.2.3: The continuity of the posterior cricoarytenoid and the thyroarytenoid primordia are apparent as well as the inferior pharyngeal constrictor in this plate.

B) N.3.2: This photograph demonstrates the myoblastic cricothyroid and the initial stages of posterior cricoid chondrification at its lower level.

C) N.3.8: The infero-ventral continuity of the prochondral cricoid is demonstrated in this photographic plate.

SH91



n.2.3

n.3.2

n.3.8

×830

Plate 20: SH101 28.5mm.CR.

A) 21.3.3 and B) 22.1.1: These photographs represent sections at the superior level of the larynx to demonstrate the myoblastic transverse arytenoid anlage

SH101

21.3.3

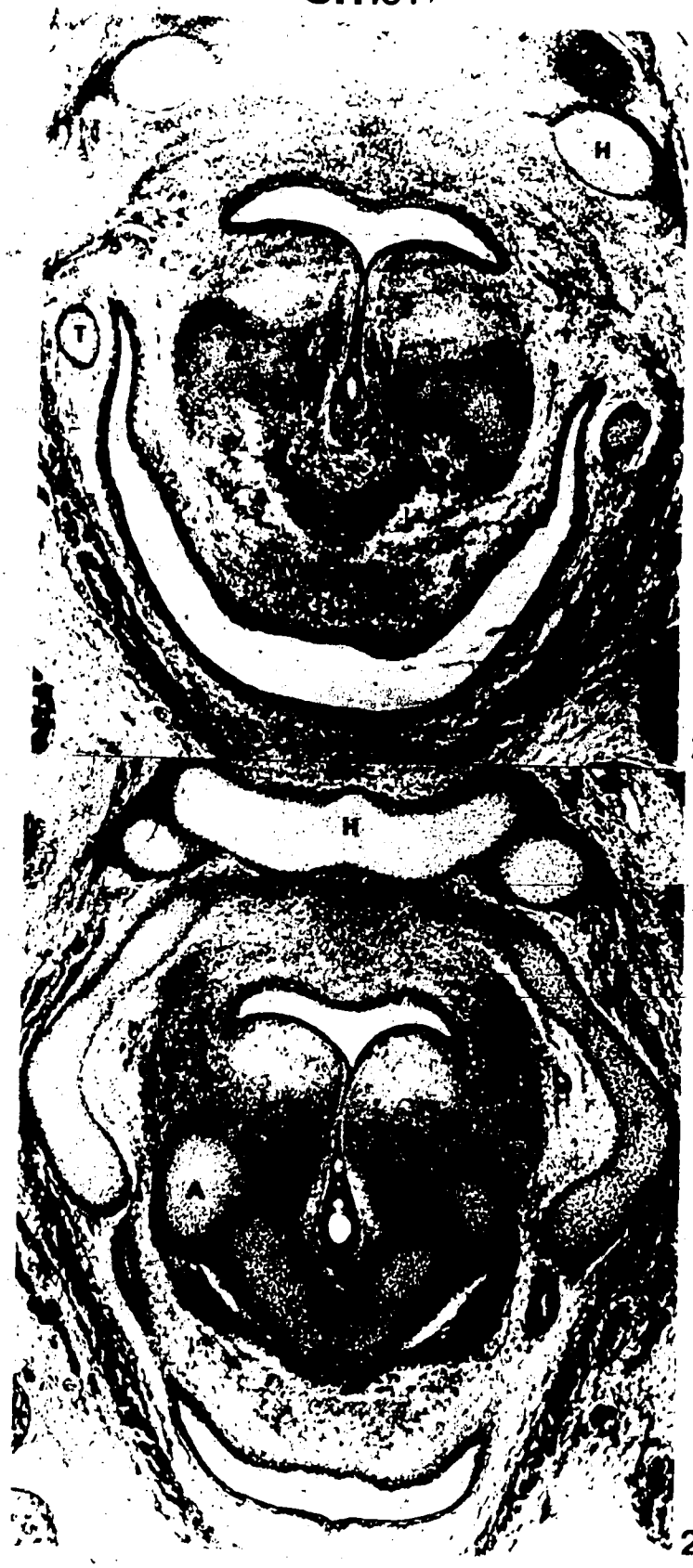
22.1.1

x630

Plate 21: SH101 28.5mm.CR.

A) 22.1.6 and B) 22.3.1: These photographs demonstrate the upper levels of the myoblastic posterior cricoarytenoid primordia and the muscular processes of the arytenoid primordia.

SH101



22.1.6

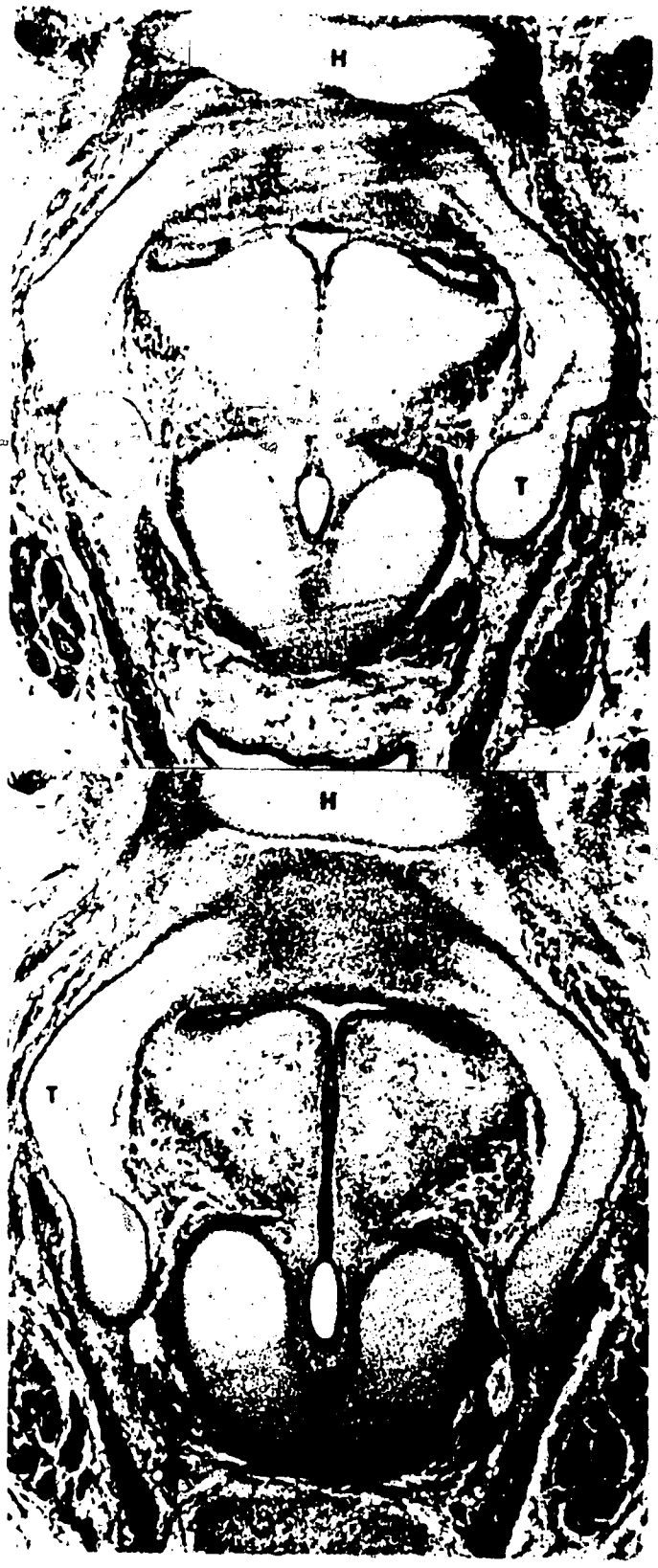
22.3.1

x630

Plate 22:. SH101 28.5mm.CR.

A) 22.4.5 and B) 23.1.3: These plates again reveal the continuity between the posterior cricoarytenoid and thyroarytenoid primordia. Posterir cricoid chondrification is complete at this level.

SH101



22.4.5

23.1.3

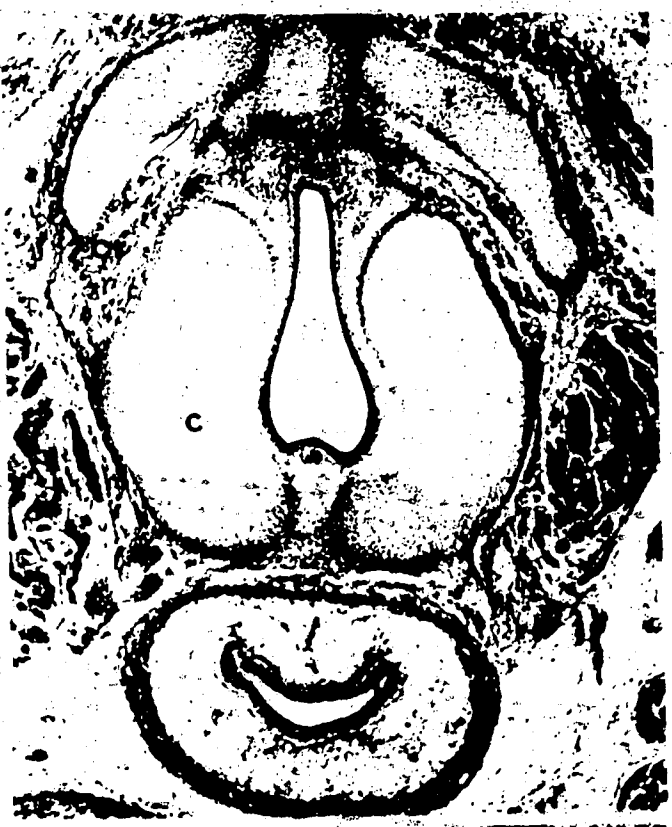
x630

Plate 23: SH101 28.5mm.CR.

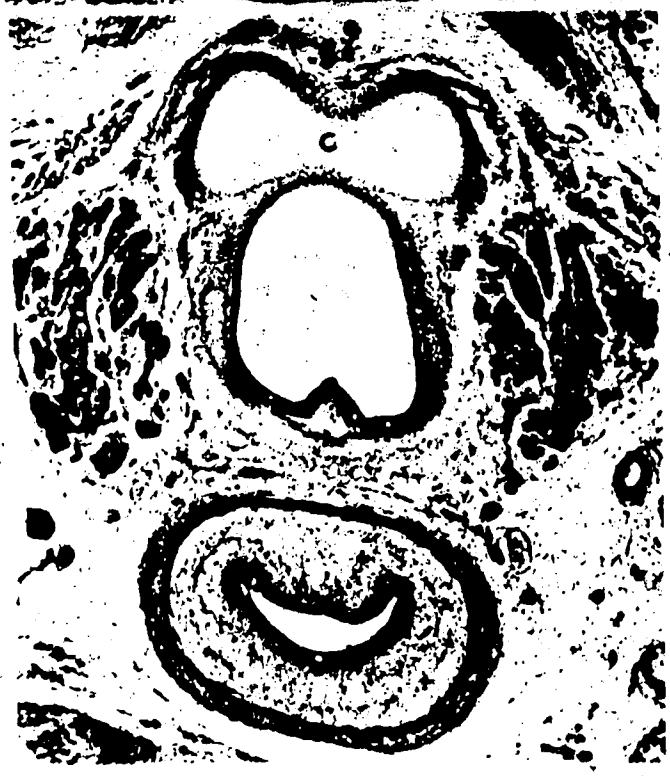
A) 23.3.3: This photograph displays the myoblastic cricothyroid muscle and the incompleteness of the posterior cricoid chondrification at this level.

B) 24.1.4: This photograph represents a section taken at the lower border of the cricoid to again demonstrate its ventral continuity.

SH101



23.3.3



24.1.4

x630

Plate 24: SH105 40.0mm.CR.

A) 9.2.2 and B) 10.1.2: Both of these photographs demonstrate the apical processes of the arytenoid primordia and the oblique arytenoid primordia.

Plate 25: SH105 40.0mm.CR.

A) 10.2.3: This photograph represents a lower section through the apical processes of the arytenoid primordia revealing the transverse arytenoid primordium.

B) 12.1.4: This plate demonstrates the position of both the lateral and posterior cricoarytenoid primordia attached to the muscular processes of the arytenoid cartilages. Anterior fusion of the thyroid laminae as well the laryngeal saccules are also apparent at this level.

SH105

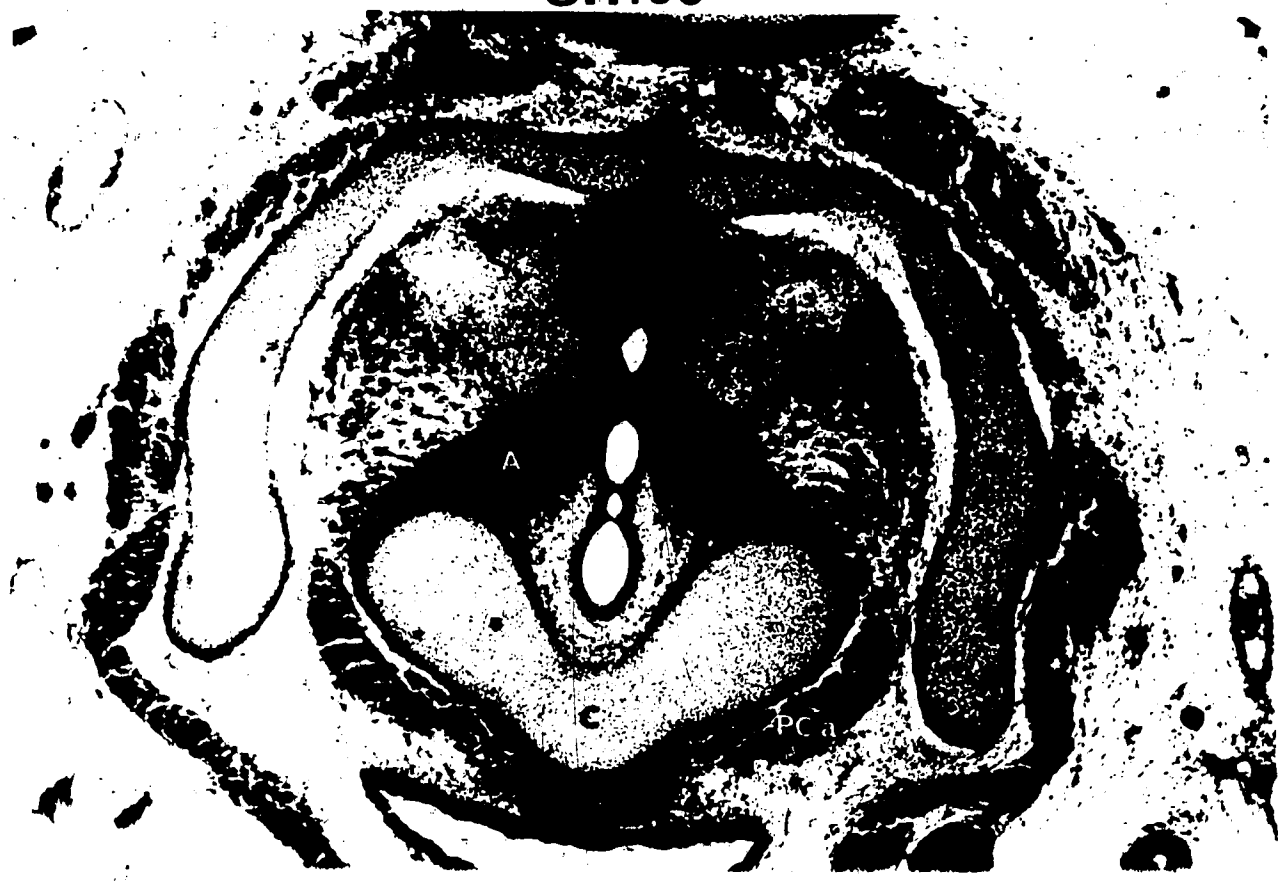
10.2.3

12.1.4

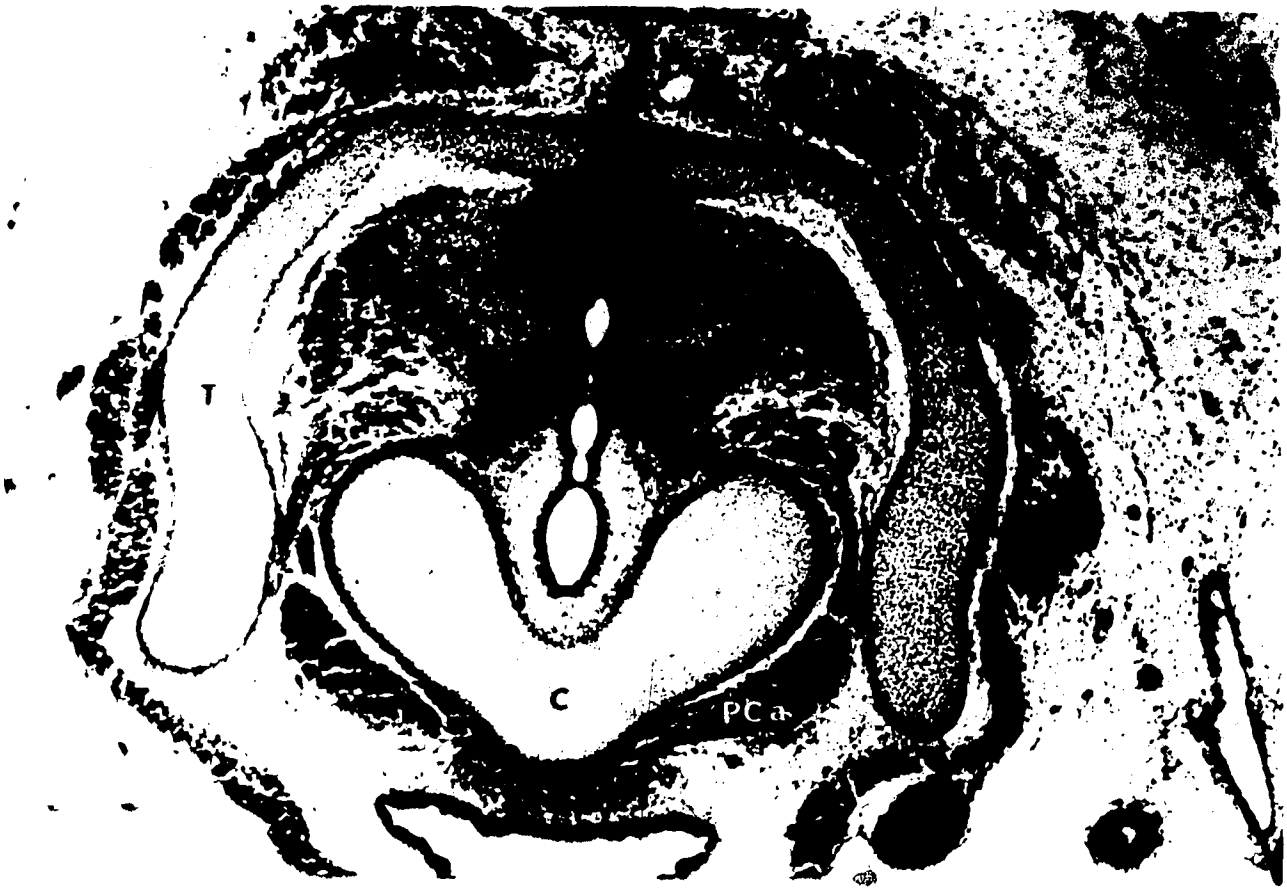
Plate 26: SH105 40.0mm.CR.

A) 14.2.1 and B) 15.1.2: These two photographs reveal the precartilage vocal processes of the arytenoid cartilages as well as the thyroarytenoid, lateral and posterior cricoarytenoid muscles.

SH105



14.2.1



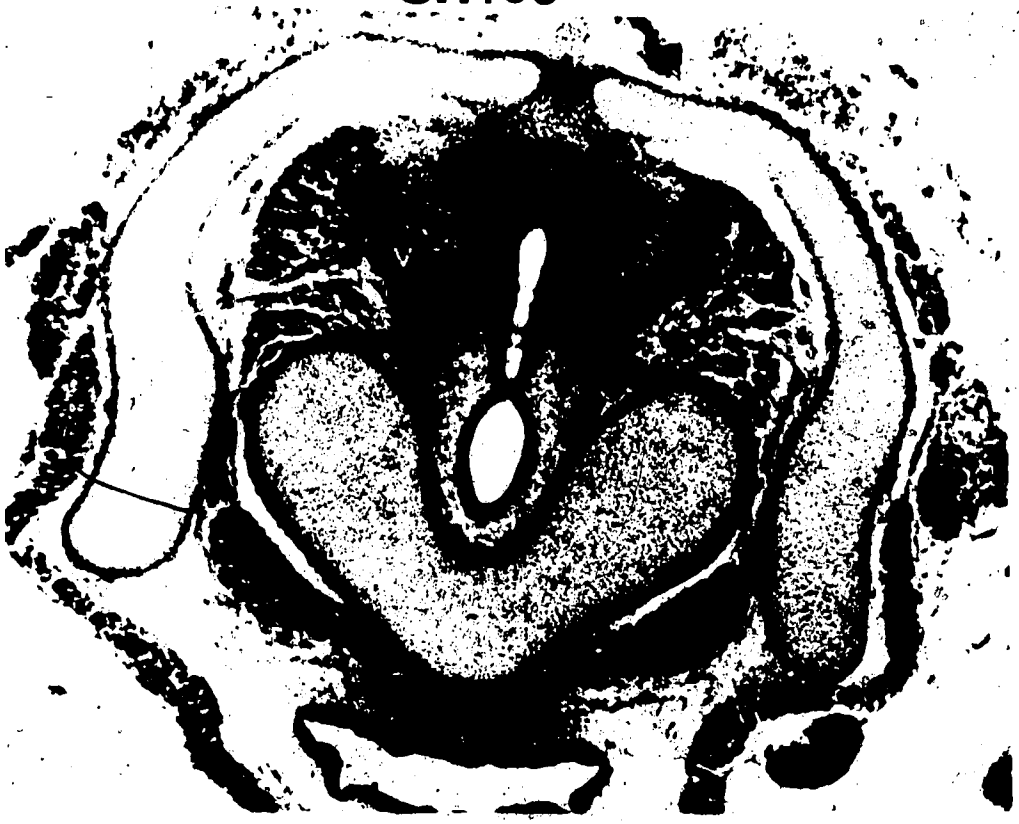
15.1.2

×405

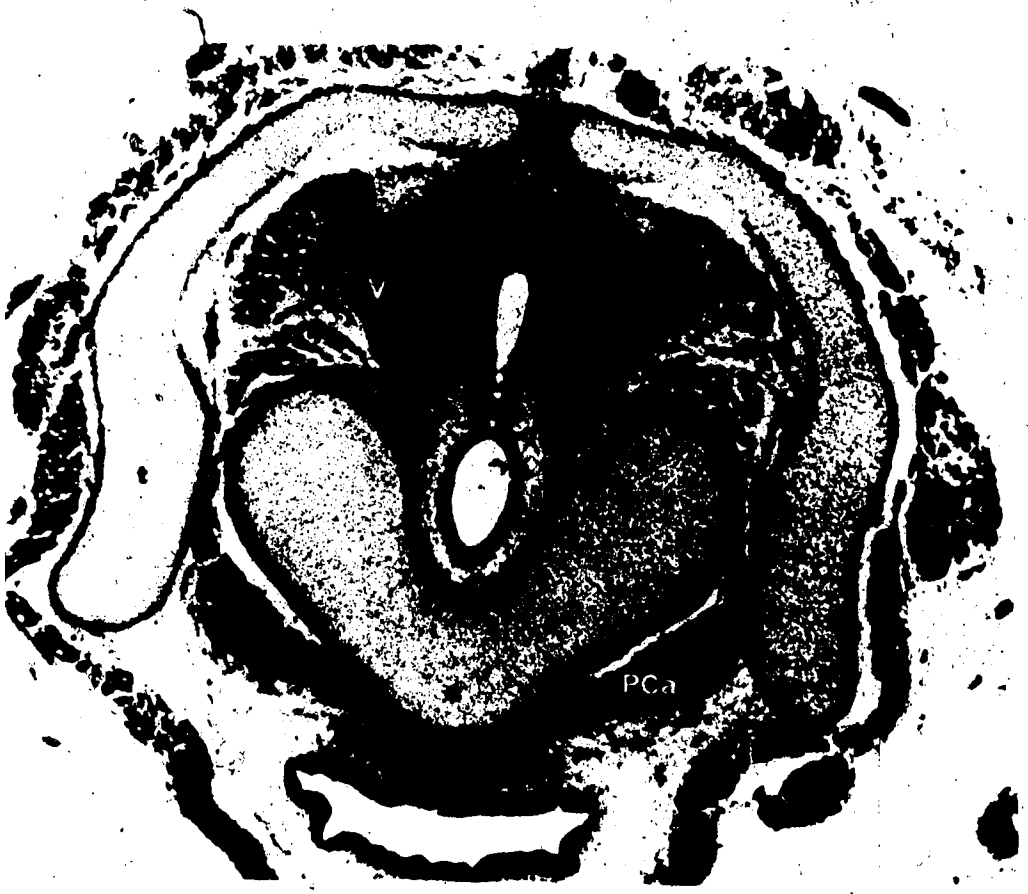
Plate 27: SH105 40.0mm.CR.

A) 15.2.1 and B) 15.2.2: These photographs demonstrate the vocalis, thyroarytenoid, lateral and posterior cricoarytenoid. Anterior fusion of the thyroid laminae at this level is incomplete.

SH105



15.2.1



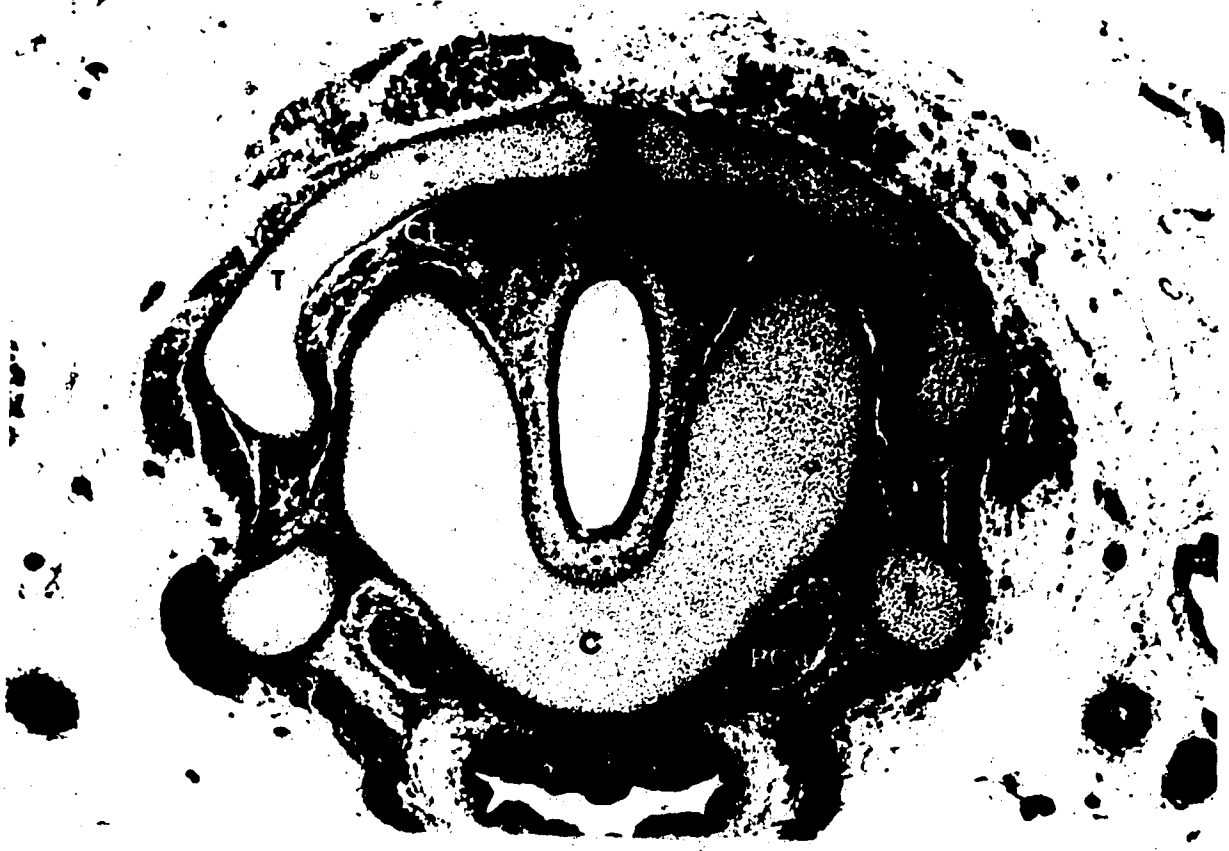
15.2.2 x405

Plate 28: SH105 40.0mm.CR.

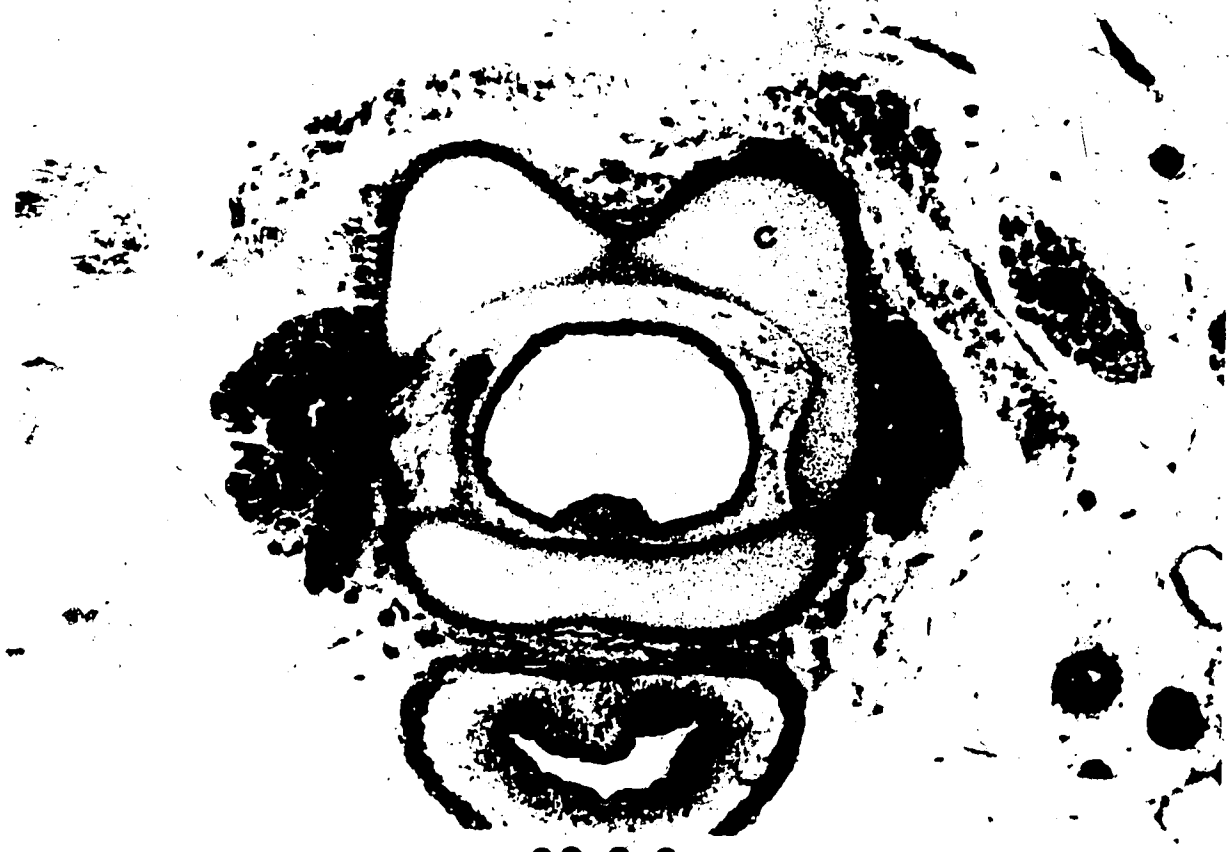
A) 17.1.1: This photographic plate reveals the separation of the cricothyroid primordia from the inferior pharyngeal constrictor.

B) 20.2.2: This plate demonstrates the completeness of posterior cricoid chondrification.

SH 105



17.1.1



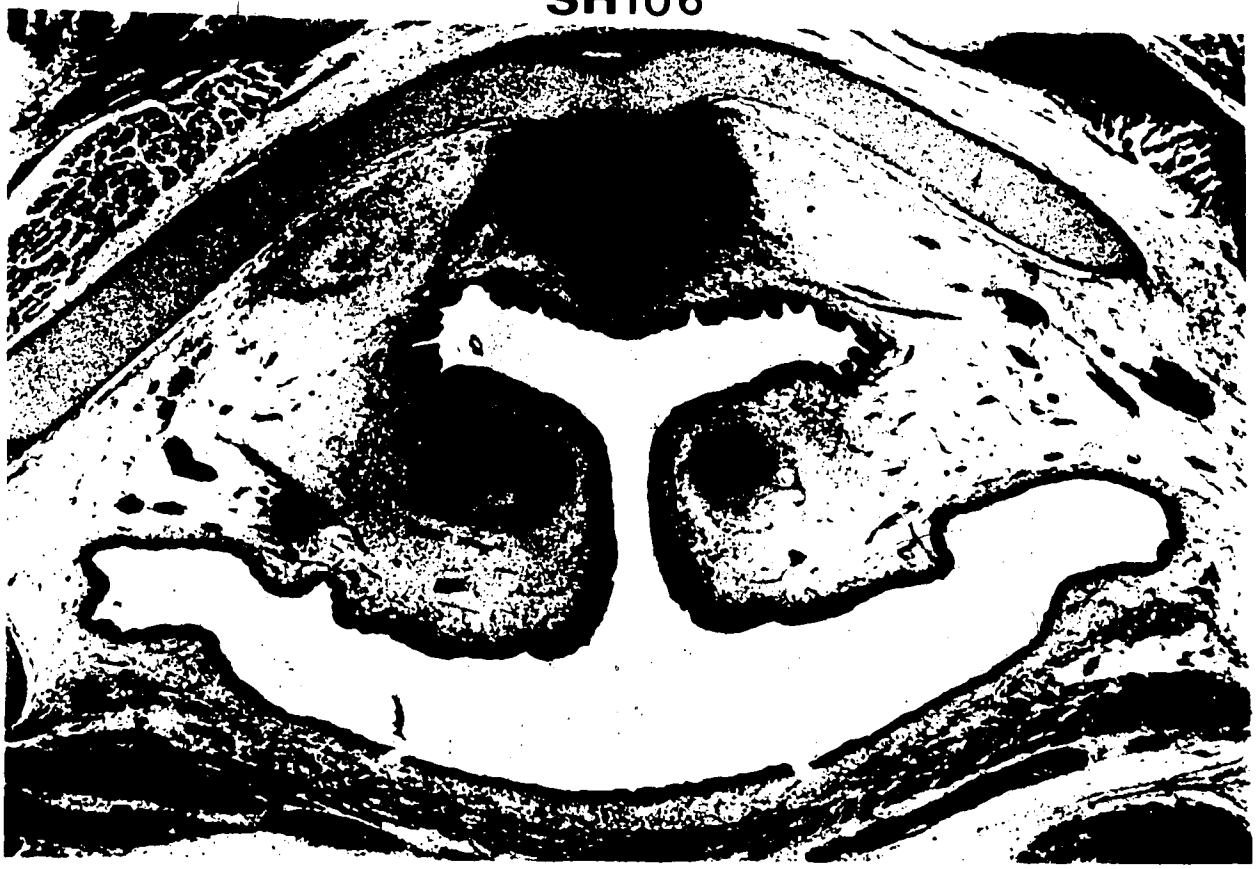
20.2.2

x405

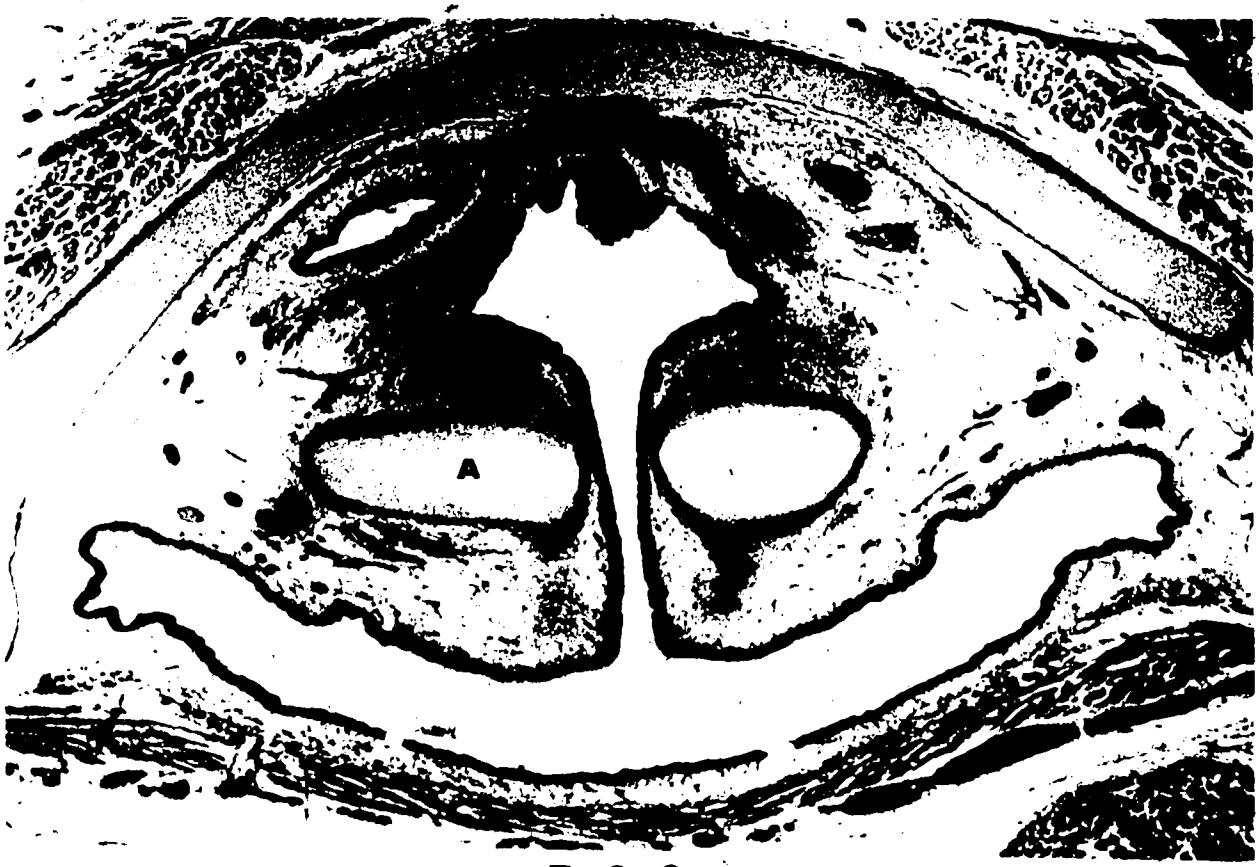
Plate 29: SH106 71.0mm.CR.

A) 6.2.2 and B) 7.2.2: Both of these photographs demonstrate the completeness of the apical arytenoid processes. The laryngeal saccules are also represented in (B).

SH106



6.2.2



7.2.2

x405

Plate 30: SH106 71.0mm.CR.

A) 8.2.2: This photograph represents a section at the upper border of the muscular processes of the arytenoid cartilages with the attachment of the transverse arytenoid and thyroarytenoid primordia.

B) 9.1.2: This photograph is essentially the same as (A) except at this level the insertion of the oblique arytenoid muscle is detectable.

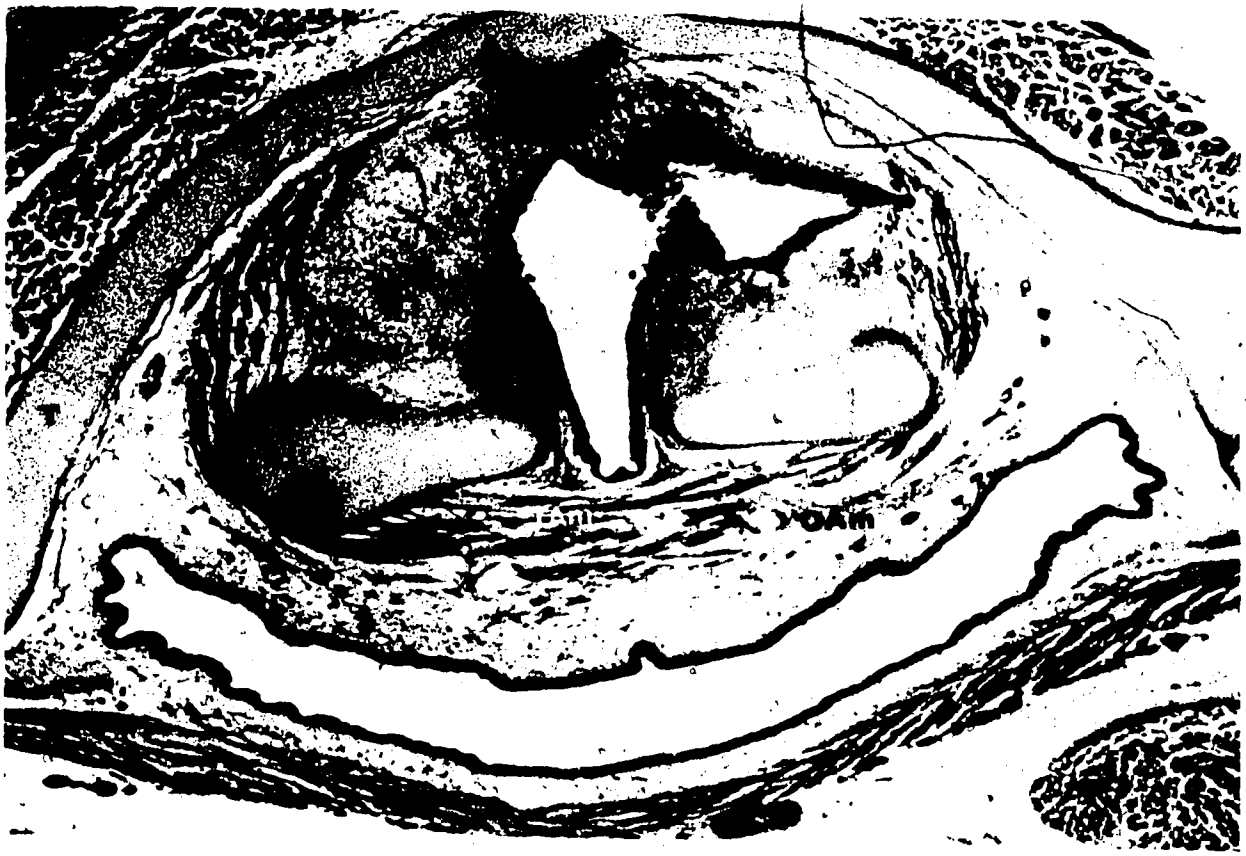
Plate 31: SH106 71.0mm.CR.

A) 10.1.3: This photograph demonstrates anterior thyroid chondrification as well as the myotube stage of the transverse arytenoid and thyroarytenoid muscles.

B) 12.1.1: Both the vocalis and the lateral cricoarytenoid muscles are evident in this photograph. Also the chondral state of the vocal processes of the arytenoid cartilages is evident.

SH106

8.2.2



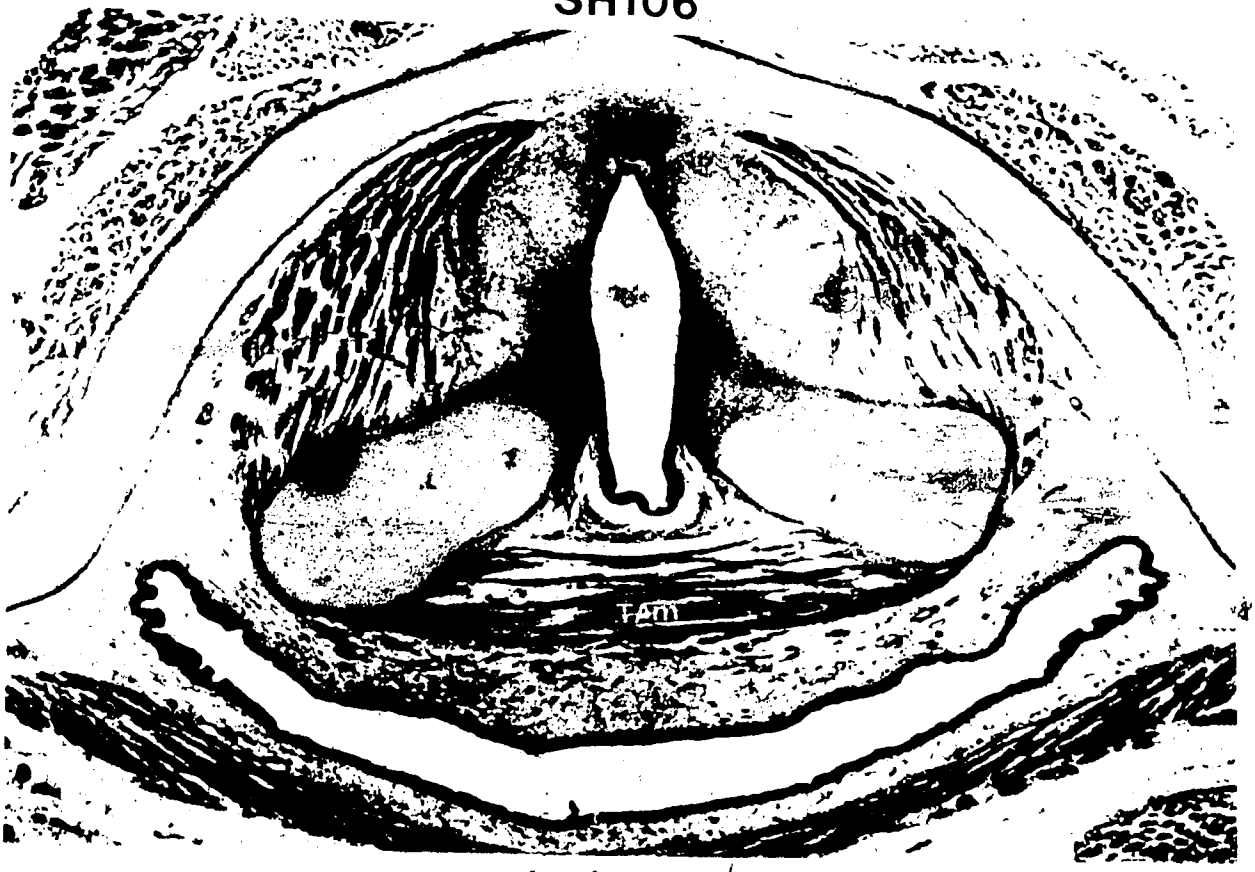
9.1.2

x405

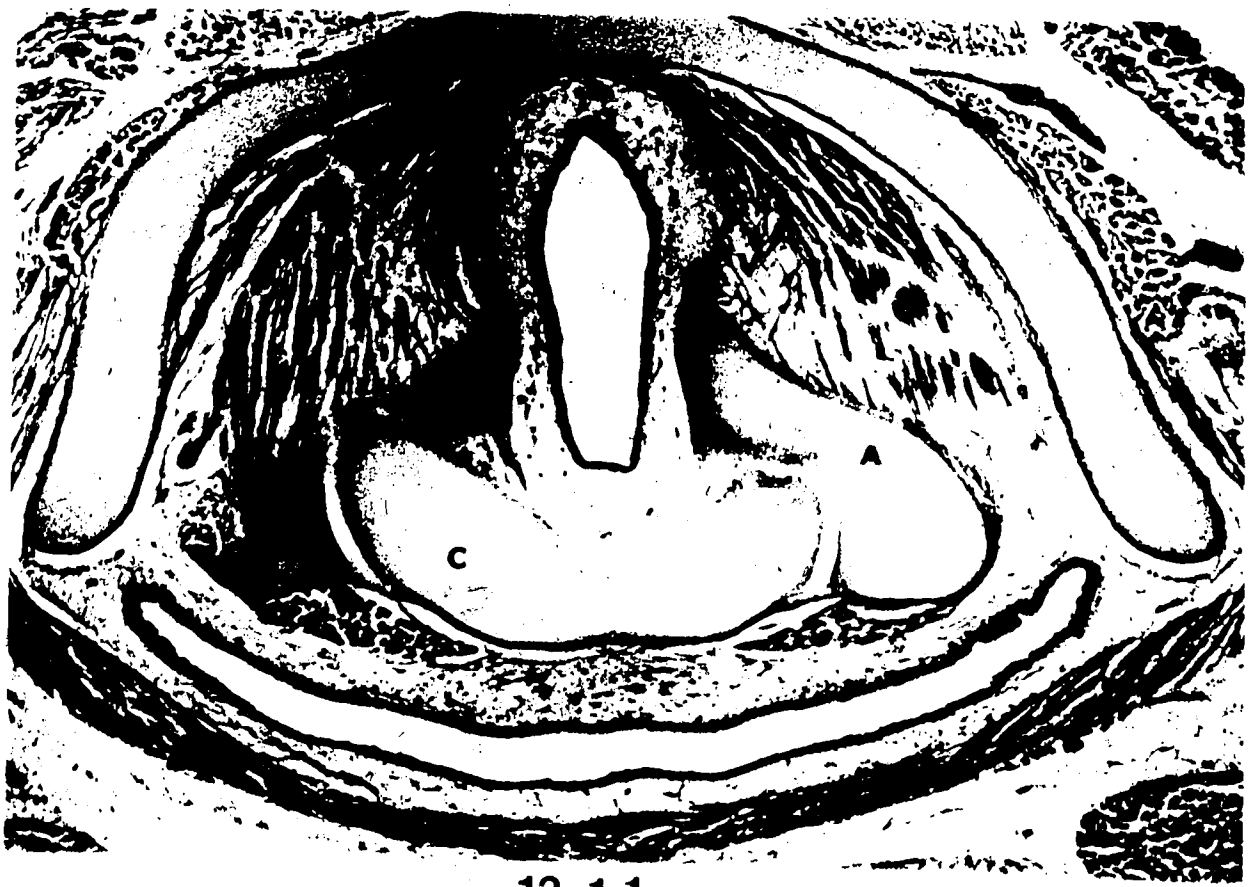
Plate 32: SH106 71.0mm.CR.

A) 12.1.3 and B) 12.2.2: In these photographs the lateral and posterior cricoarytenoid, the thyroarytenoid, the vocalis, and the cricothyroid muscles are demonstrted at the myotube stage of development.

SH106



10.1.3



12.1.1

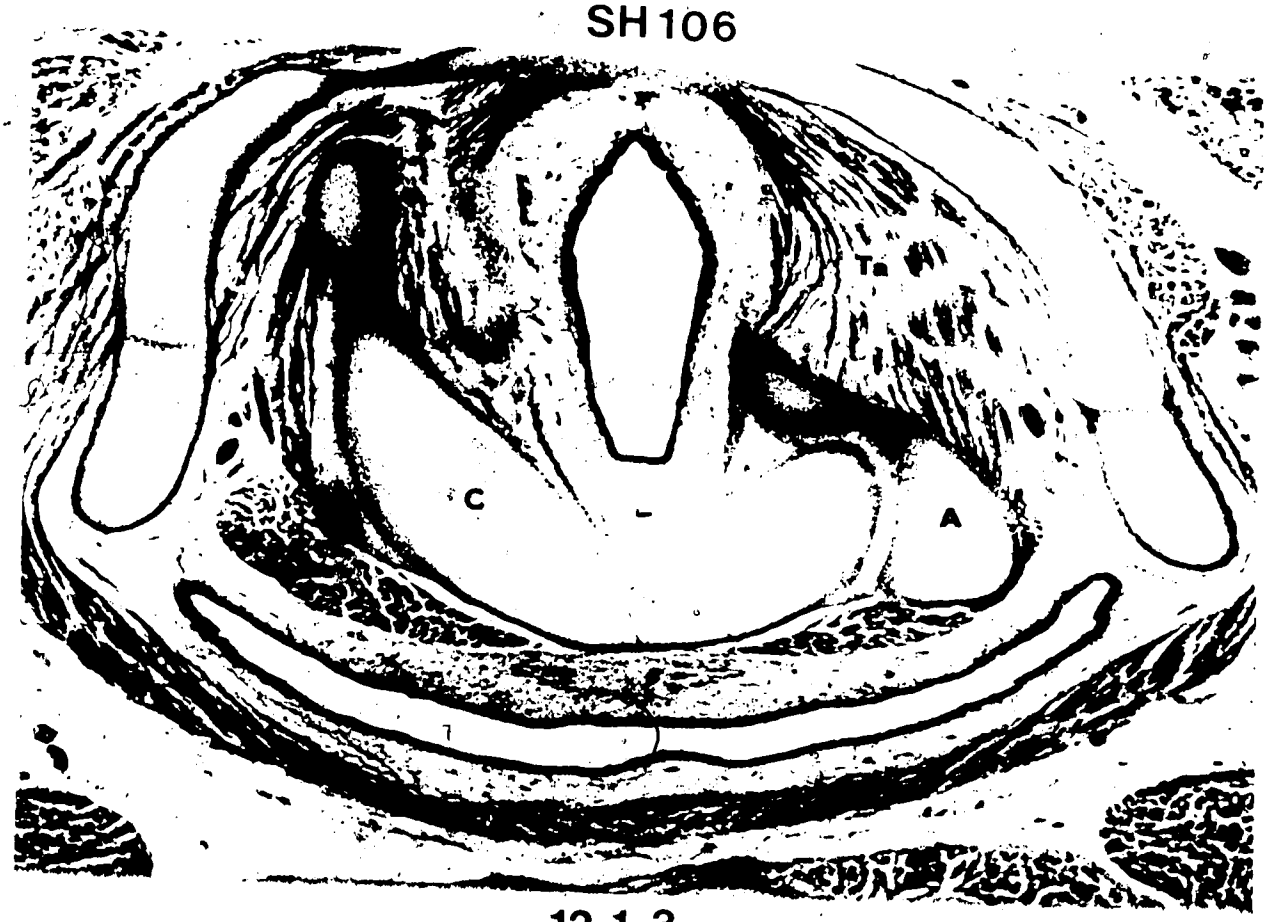
x405

Plate 33: SH106 71.0mm.CR.

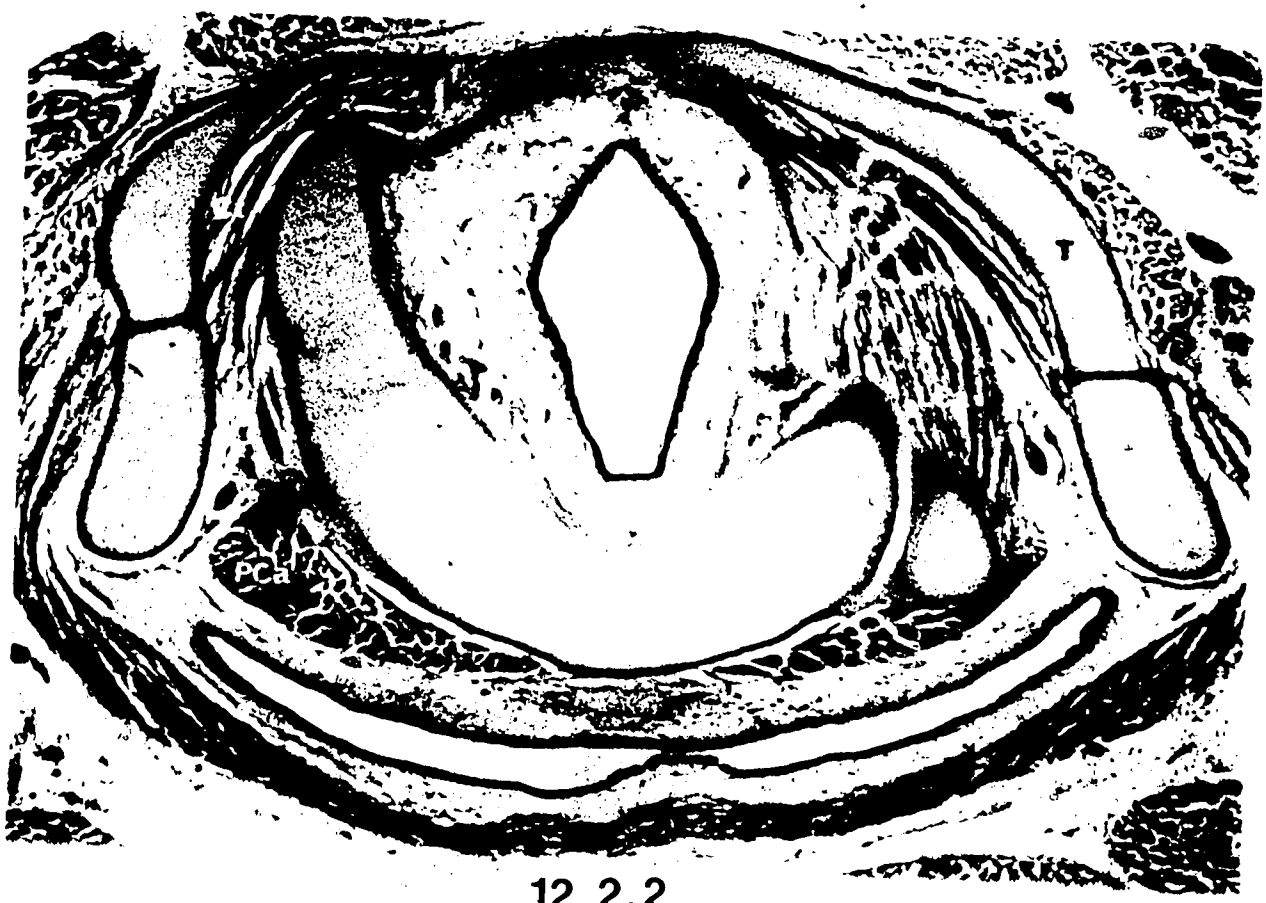
A) 12.2.3: This photograph demonstrates the position of the cricothyroid and the lateral and posterior cricoarytenoid muscles at the lower border of the thyroid laminae.

B) 15.2.2: This plate reveals complete posterior fusion of the cricoid and the continuity of some of the inferior pharyngeal constrictor fibers with that of the cricothyroid muscle.

SH 106



12.1.3



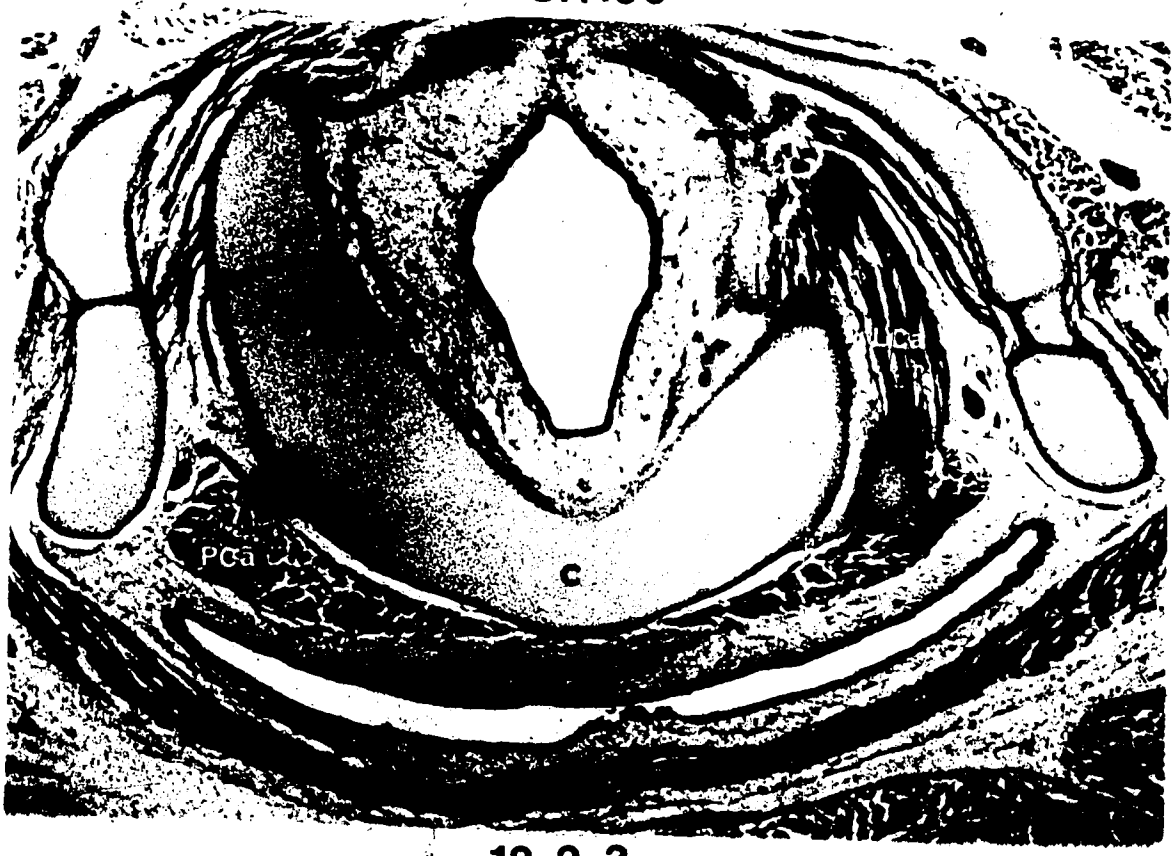
12.2.2

×405

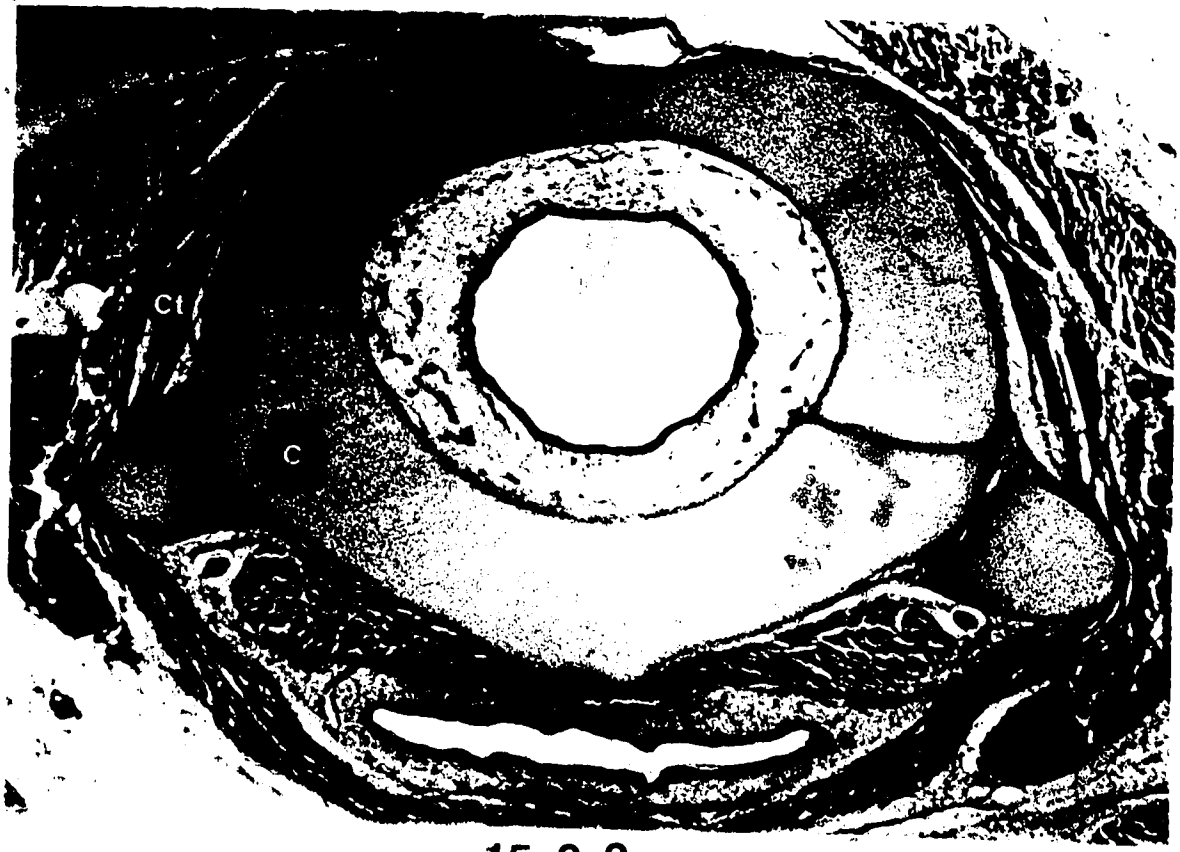
Plate 34: SH108 110.0mm.CR.

A) 28.2 and B) 35.2: These photographs demonstrate the continuity of the aryepiglottic muscle with that of the oblique arytenoid muscles.

SH106



12.2.3

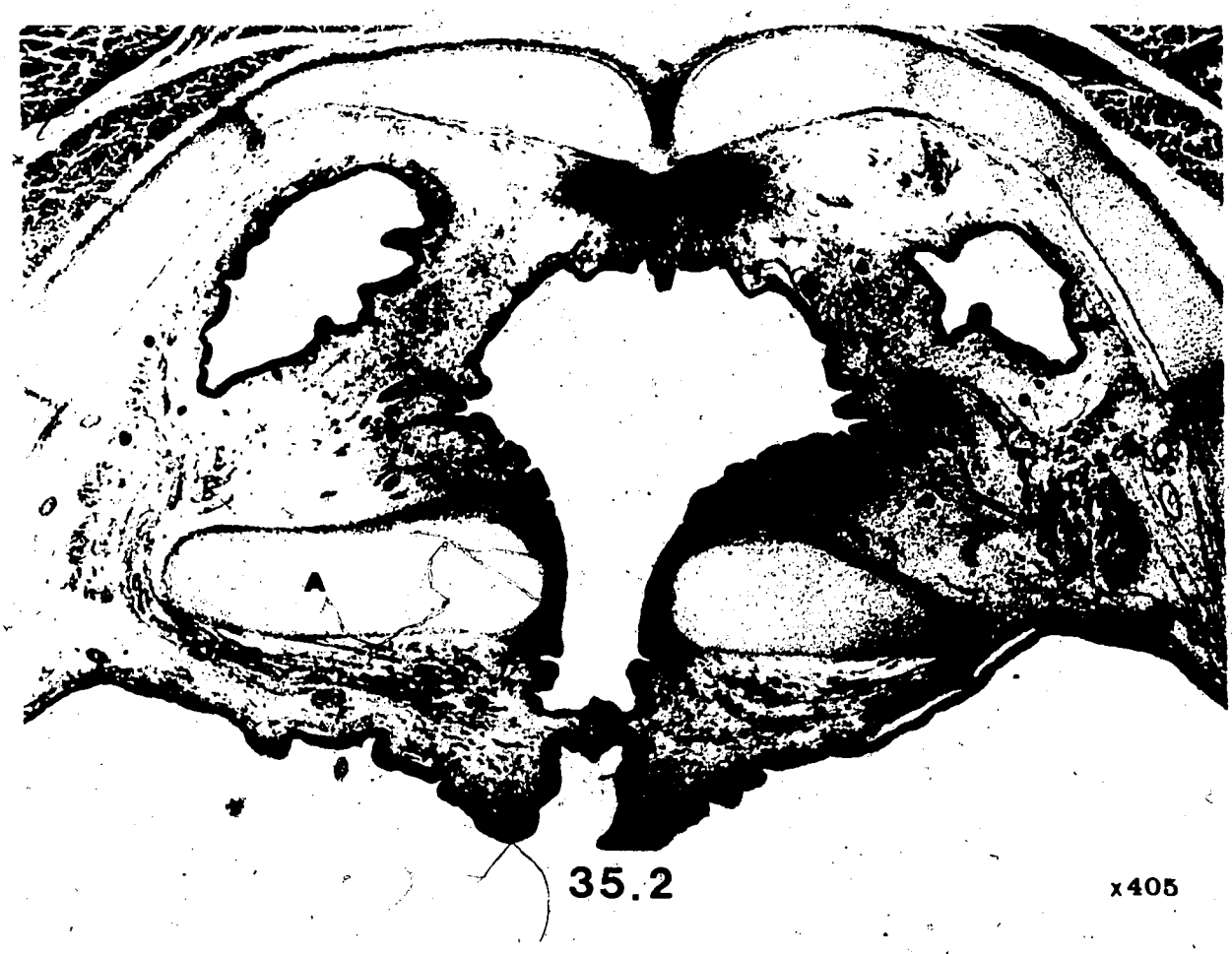


15.2.2

×405

SH108

28.2



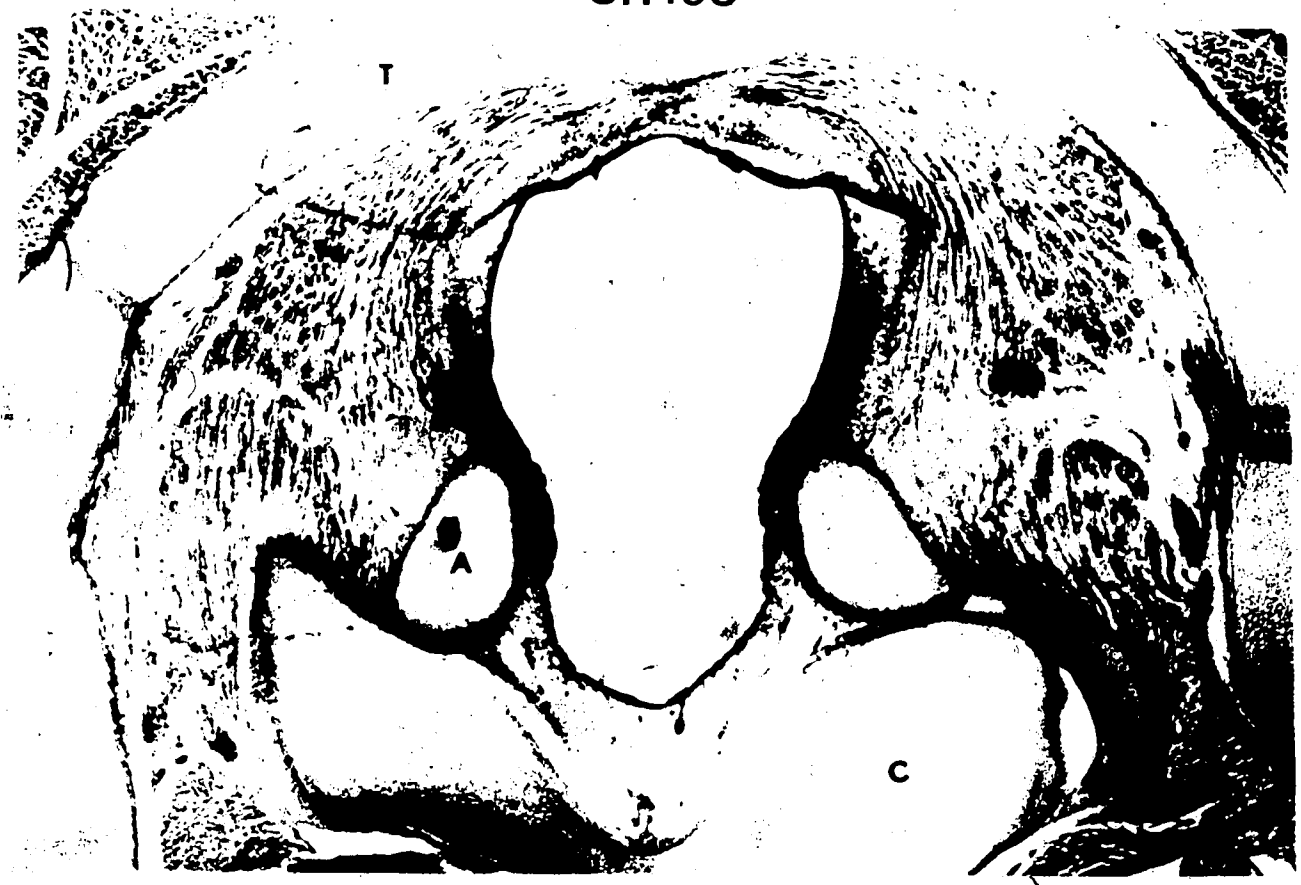
35.2

x405

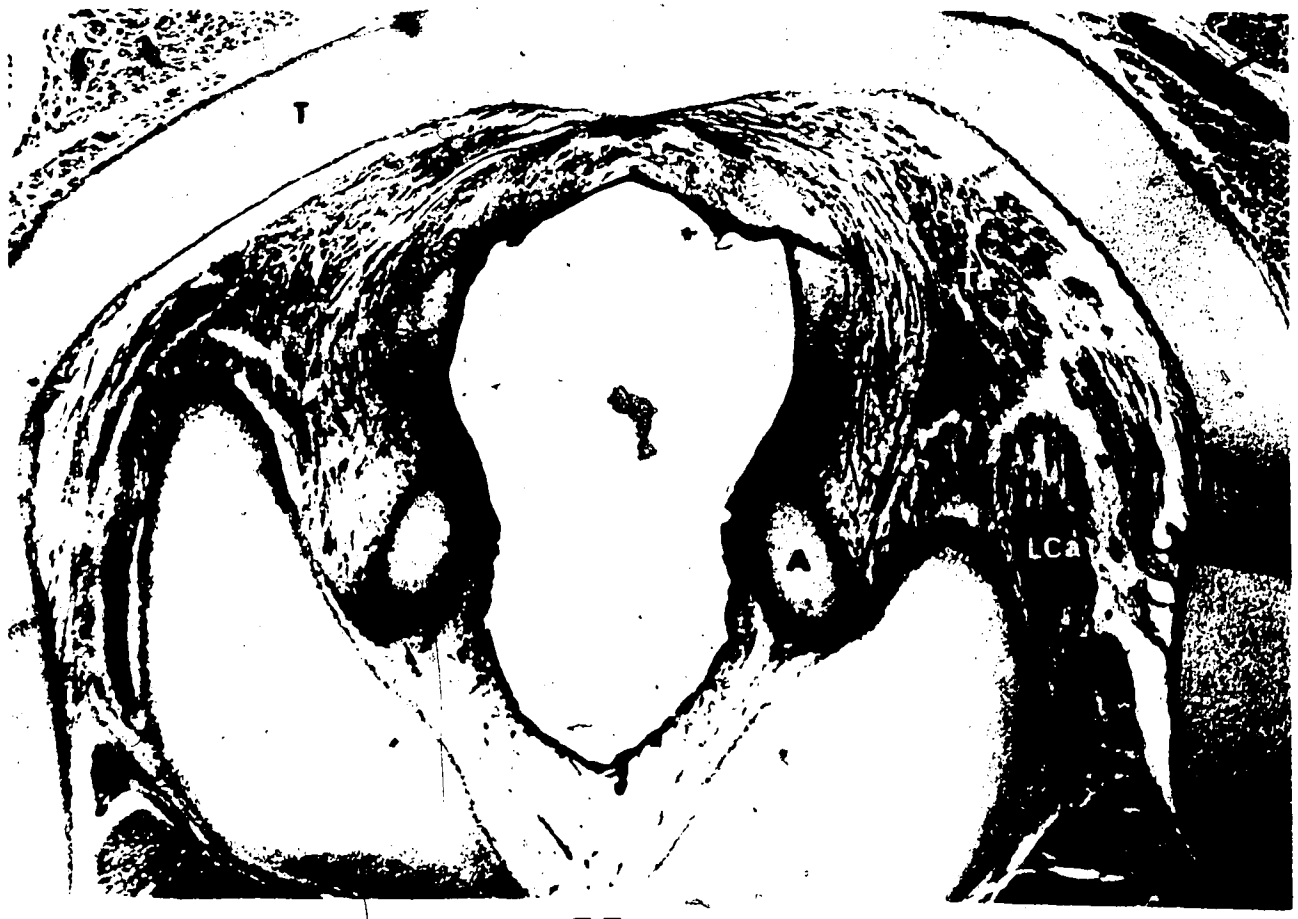
Plate 35: SH108 110.0mm.CR.

A) 53.1 and B) 55.1: Both of these photographs demonstrate the position of the vocalis, thyroarytenoid and lateral cricoarytenoid muscles at the level of the vocal processes (glottis).

SH108



53.1



55.1

x405