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THE EMBRYOLOGICAL DEVELOPMENT OF THE PHARYNGEAL REGION OF THE SHEEP

WILLIAM HENRY GERNEKE, Veterinary Research Institute, Onderstepoort

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ABSTRACT

In the Merino sheep (*Ovis aries*) four pharyngeal pouches and an ultimobranchial (postbranchial) body attached to the last pouch are formed. The development of the second and third pharyngeal pouches and of complex IV is described and discussed. Complex IV does not reach the ectoderm. A thymus II was found to exist only for two to three days. The cervical vesicle IV contributes to thymus III formation. The ultimobranchial body participates in thyroid formation while remnants of it remain as epithelial cysts in the adult thyroid. Such aberrant thyroid tissue may give rise to new follicles or undergo pathological changes if adequately stimulated (for example by iodine deficiency or in cases of increased thyroid activity such as in pregnancy). The possible role played by mesoderm or its derivatives in thyroid, thymus and ultimobranchial body formation is discussed.

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EMBRYOLOGICAL DEVELOPMENT OF THE PHARYNGEAL REGION OF SHEEP

I.—Introduction

The investigation into the development of the pharynx of the Merino sheep *Ovis aries* was initiated on the assumption that it has never been completely investigated. However, after an extensive search through the literature, it was found that before 1900 several French and German research workers (Stieda, 1881; Kastchenko, 1887; Prenant, 1894; Simon, 1896, cited by Van Dyke, 1945; Verdun, 1898) investigated certain aspects of the development. Of these, only Prenant's and Verdun's papers were obtained. After 1900 the postnatal thyroid development of the sheep was investigated by Van Dyke (1945); closure of the cervical sinus and glycogen content of pharyngeal derivatives by Scothorne (1950, 1955) and the development of branchiogenic organs by Tordoir (1935). The latter paper as far as could be determined was never published, but a micro-film of this paper was obtained after this investigation had practically been completed. As will be seen some conclusions reached in this paper differ markedly from some of those reached by Tordoir.

Cloete (1939) investigated the prenatal growth in the Merino sheep and compiled a normograph from which, by taking certain measurements, the approximate ages of foetuses (obtained from abattoirs) could be determined. Joubert (1947), Harris (1937), and Green & Winters (1945) also investigated the prenatal growth in the Merino sheep.

The pharyngeal development however has been thoroughly investigated in other animals and offers, therefore, a considerable literature. That listed in the bibliography, is by no means a comprehensive list. Kingsbury (1915 a), Badertscher (1918), Van Dyke (1945) and especially Tordoir (1935) have given excellent reviews of the literature and repetition, with exception of outstanding points, is considered unnecessary for the purpose of the present work.

The problems presented in the development of the pharynx comprise essentially the folowing:—

(*a*) Does the cervical vesicle III and/or IV participate in the formation of the thymus?

(*b*) What is the nature of thymocytes, their origin and consequently the possible function of the thymus?

(*c*) Do the ultimobranchial bodies (postbranchial bodies, suprapericardial bodies, or lateral thyroids of some other authors) also form thyroid tissue and are they provided with an inherent thyroid-forming potency?

(*d*) How many pharyngeal pouches are formed? Do the ultimobranchial bodies represent a fifth pouch? Are parathyroids III and IV always formed?

(*e*) What is the fate of the second pouch?

The problems therefore are centred around the fate of the second, third and fourth pouches. The latter are each referred to as a complex IV due to the attachment of the ultimobranchial bodies to these pouches. Each complex IV opens into the pharynx by a single branchopharyngeal duct. The first pharyngeal pouch presents no problems as it forms the Eustachian tube (and guttural pouch in some species), the epithelial lining of the middle ear cavity and the tympanic bulla. Its development is, therefore, not considered any further.

In most mammals a cervical vesicle II, and either III or IV are formed. The cervical vesicles are formed when the cervical sinus (formed when the second branchial arch grows caudad and covers branchial arches III and IV) gets " pinched off " from the ectoderm. This takes place when the second branchial arch fuses with the ectoderm caudal to branchial arch IV. Due to the presence of clefts II, III and IV and their closing plates, cervical vesicles II, III and IV are theoretically formed (Malcolm & Benson, 1940). In practice, however, it is found that the cervical vesicle II is always present, whereas cervical vesicle III or IV is usually found—III in the rat, mouse (Rogers, 1927, 1929) and ox (Kingsbury, 1935 a—"A ductus branchialis IV is thus not encountered in the calf and the cervical vesicle bears a relation to the third pouch alone ") and IV in man (Garrett, 1948). The vesicle II regresses whereas vesicle III or IV becomes attached to the ventro-lateral side of the ganglion nodosum of the vagus. (For formation of cervical vesicles in sheep see page 199.) Prior to this attachment, it (and especially the epibranchial placode which eventually forms a part of the cervical vesicle) contributes ectodermal cells ($\pm$1,300 in number) participating in the formation of the ganglion nodosum (Batten, 1957).

Norris (1938) found that, in man, the cervical vesicle IV is transformed into thymic cortex, but Garrett (1948) showed regression and disappearance to be the ultimate fate in man. Anderson (1922) states that in the calf " a cervical vesicle is formed essentially as in man and possesses the same relations to the ganglion nodosum. It forms no part of the thymus as it does in the pig ". In the rat the cervical vesicle remains fused to the cephalolateral pole of the thymus until it completely degenerates (Rogers, 1929). In the wombat and koala (Frazer, 1915, cited by Klapper, 1946) the cervical vesicle persists as a portion of the thymus which remains within the cervical region. No portion thereof is located in the thoracic cavity. In the guinea pig the thymus is mainly a cervical structure and the cervical vesicle contributes to its formation (Klapper, 1946). In the pig (Badertscher, 1915 a) and in *Tarsius* (Nierstrasz, 1912, cited by Selle, 1935) the thymus has an ectodermal and entodermal origin. In the Pacific pallid bat (Selle, 1935) only a second cervical vesicle is formed which entirely disappears—the thymus therefore being entirely entodermal. In the sheep Tordoir (1935) found no thymic contribution from the cervical vesicle—his views being in direct opposition to those found in this investigation.

In the mole Rabl (1909, cited by Rogers, 1929) reported a persisting cervical thymus derived from epithelium of the cervical sinus and an entodermally derived thoracic thymus which atrophies after birth.

Van Dyke (1945) has summarised (see his table 1) the variable fates of the ultimobranchial bodies (i.e. cyst formation and/or thyroid parenchyma contribution, formation of thymus IV, or degeneration) as interpreted by various investigators from 1880-1940. The ultimobranchial bodies are present in all orders of vertebrates with the exception of the cyclostomes (Kingsbury, 1935 a). Their absence, however, has been reported in two mammals viz. the horse (Harrison & Mohn, 1935, cited by Van Dyke, 1945) and the bat (Selle, 1935).

Regarding the fate of the ultimobranchial body in the sheep, Prenant (1894) reported cysts and some thyroid parenchyma to be formed. Van Dyke (1945) lists the following conclusions reached: Wolfler (1880), Stieda (1881), De Meuron (1886) and Kastchenko (1887) all believed that the ultimobranchial body in the sheep gave rise to thyroid parenchyma. It therefore constituted the " lateral thyroid " and was considered to have inherent thyroid-forming properties. This is one of the opinions maintained by various investigators in connection with the ultimobranchial body. Others considered it to be a ductless gland with an undeter-

mined function. The last and most probable possibility is that it is considered to be indifferent tissue (Kingsbury, 1914) but becomes induced by the thyroid (due to their intimate association) to form thyroid parenchyma (Rogers, 1927). Parts of the ultimobranchial body occasionally not included in the thyroid (branchopharyngeal cord) may give rise to small cysts or even to a thymus IV (Klapper, 1946).

In the golden hamster the ultimobranchial body is associated with pouch III (a pouch IV as in the rat not being formed) and also forms a small contribution to the thyroid (Klapper, 1950).

In mammals we mostly have four pharyngeal pouches formed. The ultimobranchial body, which in humans is considered to be a possible fifth pouch, is attached to the fourth pouch (therefore complex IV) in most mammals. In the sheep Tordoir (1935) considered the ultimobranchial body to be a fifth pouch due to the presence of a capillary representing a fifth aortic arch and a small branch of the vagus in the mesenchyme between pouch IV and the ultimobranchial body. He, however, also preferred the term caudal complex introduced by Tandler in 1909. In the rat (Rogers, 1927) only three pouches are formed with the ultimobranchial body attached to the third pouch. Only a parathyroid III is formed in the rat due to the failure of pouch IV to develop. The rat in this respect, therefore, forms an exception to the more general statement of Nelsen (1953, p. 880). " In those species having but two parathyroids, it is probable that their origin is from the fourth branchial pouches ". Tordoir (1935) makes the statement that only parathyroids III are present in the pig, mole, guinea-pig, rat, field mouse, " spitsmuis " and " zeehond ". In the Pacific pallid bat Selle (1935) found that only a parathyroid IV is formed and no parathyroid III.

Earlier literature on the subject of pharyngeal development has been mainly or purely descriptive. In the last number of years considerable experimental embryological data have become available and an attempt will be made to incorporate as much as possible of the available and applicable data as, in the opinion of the author, will lead to a better and more logical understanding of the intricate morphological and physiological changes incorporated in pharyngeal development. Experimental data, however, in respect of the latter remain difficult, if not impossible to obtain. This is due to the manner of mammalian development (within foetal membranes in the uterus) and the early anlage of the pharyngeal region, making excision experiments of the various pharyngeal derivatives as yet impossible to perform. Recourse must, therefore, be had to tissue cultures and transplantations of early organ-anlages to the new-born.

II.—Materials and Method

To obtain some of the embryos needed for this study a number of ewes were mated and slaughtered after the embryos had reached the required ages. For practical purposes fertilisation was considered to coincide with the last mating (although the error may be considerable) and the age of the embryo was calculated as from that time. It was for instance found that embryos which, according to the data of mating, were 15, 16 and 17 days old were almost at identical morphological stages of development. This could be explained by the fact that fertilisation hardly ever coincided with the last mating or perhaps by the phenomenon of heterochrony or " unequal rate of development in parts " (Balinsky, 1960, p. 249).

Additional embryos and foetuses were obtained from the Pretoria Municipal Abattoir and their ages determined according to Cloete's (1939) normograph. The ages of the embryos and foetuses are therefore to be considered merely as a guide and not as being absolutely correct.

Embryos were fixed in 10 per cent formalin, Zenker's or Bouin's fluid, embedded in paraffin wax, with a thin, smooth and straight strip of testes as a base line, sectioned serially, transversely or sagittally at 10 μ and stained with the standard Mallory Azan or H. and E. method. Zenker's fixation and H. and E. staining proved to be the most advantageous.

A number of ceresine wax reconstructions of some aspects of pharyngeal development were made (Fig. 1 to 3, 6 and 7). Using either a camera lucida or a projection microscope, tracings at 50x, 100x and 200x magnification were made. With serial sections at 10 μ these reconstructions were relatively simple, as each section represented a wax sheet 0·5 mm, 1 mm or 2 mm thick depending on the magnification used. Wax sheets of the desired thickness were rolled out, the tracings cut out on the wax sheet and the sheets so obtained joined consecutively together with the previously incorporated base line (the thin strip of testes) as a guide.

In the reconstruction the base line would then be the only straight, vertical line. Smoothing down with a heated knife, after removal of the base line (which by now had served its purpose of guiding morphological curves in the reconstructed embryo) completed the reconstruction.

III.—The Development and Fate of the Second Pharyngeal Pouch

Observations

The anlage of the second pouch is formed in the sheep embryo at 16 days of age (14 somites). At 17 days it is well-formed, without any caudal demarcation. As no third pouch has as yet been formed, a gradual taper towards the posterior part of the foregut is obvious. No contact of entoderm with ectoderm has been made.

At 18 days of age three pharyngeal pouches and the anlage of the fourth are formed. Only the first three have made ectodermal contact. These three have already formed branchial clefts (Fig. 1, 9). Of these the second is deepest showing a rather thin pharyngeal membrane. This membrane is seen to be ruptured in most embryos just older than 20 days. A similar occurrence has been described in the calf by Anderson (1922) and Kingsbury & Rogers (1927), in the guinea pig by Klapper (1946) and in the sheep by Tordoir (1935). The rupture probably takes place due to mechanical tension exerted on it by the overgrowth of branchial arch II during the formation of the cervical sinus. When ruptured, its nature is such that it cannot be considered as an artefact.

The second and third aortic arches are largest. Number one very soon shows a decrease in size. It eventually regresses (see Fig. 1).

At about 23 days (9·5 mm) the pharyngeal membrane II (closing plate) is reformed and the branchial cleft II, after formation of the cervical sinus, is changed to form a branchial duct (Fig. 7, Bd. 2). This duct connects its slightly dilated inner end—the cervical vesicle II in contact witth the pharyngeal membrane II—with the cervical sinus.

Pouch II at 26 days (12 mm) shows two caudoventral projections (Fig. 7, 14):—

(*a*) One, slightly larger, directed caudolaterally (Fig. 7, Clp.) and attached to the cervical vesicle II (Fig. 14), becomes gradually severed in older embryos from the caudal extremity of pouch II by the loop of the lingual branch of the glossopharyngeal nerve as it passes over the caudomedial surface of pouch II, and

(*b*) another smaller pouch present as a typical caudoventral diverticulum (Fig. 7, Vd) which becomes separated from the larger one and from the pharynx also by the lingual nerve. The proximal portion of this pouch remains attached to the pharynx after severance of its distal (caudal) extremity (the latter is described lower down). This proximal portion then becomes a blind pocket, the cavity of which becomes absorbed in the expansion of the pharynx.

The glossopharyngeal nerve passes down along the dorsal surface of pouch II, curves inwards around the caudomedial end of pouch II and passes craniomedially along its ventral surface from where it innervates the anterior part of the tongue (Fig. 7).

As the cervical region enlarges, the above caudolateral pocket of pouch II becomes drawn out due to its attachment by means of the pharyngeal membrane to the cervical vesicle II. The cervical vesicle II and its branchial cord (earlier duct) are also extended. At 26 days already, this cord, cervical vesicle and pocket show rupture and fragmentation (Fig. 15). These regressive changes are initiated in embryos of 24 and 25 days of age. It appears to be a fairly active destruction as phagocytes surrounding the cord are filled with cell debris. Some of these ectodermal and even entodermal cells may survive the retrogressive changes and remain buried in the neck. Subsequently, following the necessary stimuli (pathologic, viral, genetic or unknown) they may give rise to tumours, cysts or even fistulas (Malcolm & Benson, 1940).

At 28 days no visible trace of the caudolateral pocket or cervical vesicle II and cord can be found. It has therefore completely regressed.

Caudo-medial to the above pocket, the caudo-ventral diverticulum [distal, extremity of (*b*) above] can be seen as an isolated cord of cells associated with a small blood-vessel from the third aortic arch. It has lost its contact with pouch II and therefore actually represents a vestigial thymus II. No trace of it can be found at 28 days. It is seen to be best developed at 25 days. Therefore, although the anlage of a thymus II is virtually formed, it undergoes a very early regression (see page 197).

Due to the dorso-ventral compression and the elongation of the pharynx, only a ventral groove remains of pouch II. This is separated from an anterior groove of the lingual fold by the tonsillar fold (" plica tonsillaris " of Kingsbury & Rogers, 1927). The palatine tonsil then originates from the anterior and posterior tonsillar fossae so formed (Fig. 11, 12, 13)—(Kingsbury & Rogers, 1927). The epithelia of these anterior and posterior fossae grow out ventrolaterally and develop branches. Thus are formed the sinuses of the palatine tonsil. Only the posterior fossa represents the ventral region of pouch II. The condensed mesenchyme about the region of the hyoid bone contributes to the tonsil. This is due to an epithelio-mesenchymal relationship. The separate fossae tonsillares fuse to form a single sinus tonsillaris as is seen in an embryo of 34 days old (Fig. 13). The fossae start growing out at $\pm$ 34 days of gestation.

The development of the tonsil was followed up to 58 days at which time the outpocketings had increased in number and become slightly deeper. No formation of lymphocytes is encountered at that age. Later on in the development mucous glands are formed as cordlike epithelial invaginations. The outpocketings become hollow and the lining stratified squamous epithelium becomes invaded by lymphocytes to such an extent that the epithelial cells actually resemble the reticular cells—simulating therefore thymus structure. Occasional structures resembling Hassall's corpuscles are encountered (Kingsbury, 1928).

2. Discussion

Scothorne (1957) shows in his Table I the occurrence of thymus II in vertebrates. Being present in fishes, amphibia, lower reptiles, some birds and some marsupials it is to be expected that in higher mammals where it is absent in adults, it will either also be absent in the embryo or just present as an abortive structure. Signs of its disappearance are already shown by poor development in some birds and marsupials.

In the sheep it is present as an abortive structure. The vestigial cord representing a thymus II and showing a phylogenetic relation with lower vertebrates is present only for two to three days after which complete regression takes place. No evidence of a thymus II in higher mammals was encountered in the literature. Tordoir (1935) does not mention it either.

Pouch II in mammals is completely an abortive structure. The older concept that pouch II forms the palatine tonsil has been ably waylaid by Kingsbury & Rogers (1927). They definitely state that, " only the posterior fossa can in any way correspond to the second pouch, and indeed only to its ventral portion, while the ' sinus ' appears as a new feature in the pharyngeal topography. A complete linkage of palatine tonsil and second pouch appears, therefore, to be without justification in fact ".

A similar situation is present in the sheep. The tonsillar sinus is formed secondarily as a fusion of two separate invaginations and no trace of the cavity of pouch II remains. In the formation of the tonsillar sinus the underlying mesenchyme and the development of the soft palate must play a role. The factors involved are fully discussed by Kingsbury & Rogers (1927) for the ox. In the sheep, being a ruminant with the same relations, the same will apply. They " regard the tonsillar fossae and sinus as determined by the growth transformations characteristic of the region, and the tonsil, an expression of an altered epithelio-mesenchymal relation ".

Here the effect of the mesenchyme on the epithelium is seen—stimulation to cordlike proliferations and subsequent reticulation of the stratified squamous epithelium by invading lymphocytes. Here also as in the thymus (see later) the epithelial cells stimulate formation of the lymphocytes from the mesenchymal cells.

The palatine tonsil in conjunction with the thymus must probably act as an early source of lymphocytes which are then responsible for immunity and other reactions in the foetal body (see later).

IV.—The Development and Fate of the Third Pharyngeal Pouch

(1) *Observations*

The third pharyngeal pouch develops as a lateral evagination of the pharynx of the sheep embryo between 17 and 18 days of age. In an embryo of 18 days pouch III is well-formed, has reached the ectoderm and shows a slight caudo-ventral pocket—the early anlage of thymus III. (Fig. 1, 2. 3.)

In a slightly older embryo (20 days 17·5 hours) a dorso-lateral protrusion in contact with the branchio-pharyngeal membrane is formed, probably due partly to compression by the third aortic arch which then comes in intimate contact with the anterior dorsolateral surface of pouch III. This dorsolateral epithelium of pouch III shows a thickening—the anlage of parathyroid III (Fig. 31, 32). Adjacent to this thickened epithelium a slight capillary plexus is present which originates from the third aortic arch. The thickened epithelium soon presents (23 days) a slight evagination with a small central cavity in the region just lateral to the fourth aortic arch, i.e. on its posterolateral surface. Proliferation to form branching and anastomosing cell cords ensues and vascularisation from the capillary plexus takes place. The central cavity cannot be identified in further stages and therefore must be obliterated very soon after formation.

The development of the parathyroid so initiated is especially rapid between 24 and 26 days. As the parathyroid cells are strongly eosinophilic from the start they are easily identified. In an embryo of 26 days the parathyroid was found to be a well-developed C-shaped body with its anteroventral border curving around the third aortic arch. In the ox it also " conforms to the shape of the carotid about which it fits " (Anderson, 1922). In the sheep its dorsal border is separated from the ganglion nodosum of the vagus by only a thin layer of reticular connective tissue. The anterior laryngeal branch of the vagus (with ganglion nodosum just above point of branching) passes dorsomedially to the third pouch (23 days) (compare Fig. 7) directly adjacent to the anlage of the parathyroid III. In earlier stages the ganglion nodosum comes close to the ectodermal placode postero-dorsal to the pharyngeal membrane III (Batten, 1957) (Fig. 31). The ganglion nodosum of the vagus, its anterior laryngeal branch and the aortic arch III and its capillary plexus are closely associated with the anlage of the parathyroid and may therefore be considered to play a role in the induction of the parathyroid.

The anlage of thymus III is flattened cranio-caudally between aortic arches III and IV (20 days 17·5 hour embryos) (Fig. 1, 6) and is situated immediately lateral to the aortic sac, extending caudally just beyond the fourth aortic arch (Fig. 27). At 24 days its most ventral point is directed caudo-medially (Fig. 3). It is in close contact with the mesenchyme surrounding the aortic sac and fourth aortic arch and is probably actively drawn caudally as the heart migrates caudad. The caudal growth of the thymus anlage appears therefore to be governed by the caudal migration of the heart. At 25 days of age the fourth aortic arch indents the caudo-dorsal point of the thymus anlage. It actually fits around the arch antero-ventrally and thus shows its intimate contact with the mesenchyme around the aortic arch.

Severance of the branchio-pharyngeal duct from the pharynx occurs at about 28 days (Fig. 32, 33). This severence is facilitated by the anterior laryngeal nerve which curves around it caudally (Fig. 7). The parathyroid-thymic cord, however, is maintained.

As further development of the thymus cord to some extent is influenced by the presence of the cervical vesicle IV its formation will first be described.

Although complex IV (see later) never comes in contact with the ectoderm, a small branchial cleft IV is formed (Fig. 1, 31). The ectoderm on both sides of this cleft thickens as if responding to an inducing influence from either complex IV or ganglion nodosum. This forms the vagal placodal ectoderm (epibranchial placode of vagus) (Fig. 31) and, according to Scothorne (1955), can be sharply distinguished from the surrounding skin ectoderm at the 12 mm stage ($\pm$25 days) by the complete absence of glycogen.

Rapid enlarging of the heart directly caudal to the branchial area, enlarging of branchial arch II, attachment of ectoderm to pharyngeal pouches II and III and of ganglion nodosum to vagal placodal ectoderm cause the formation of sunken cervical vesicles II and IV. The latter cervical vesicle is, therefore, formed by the submergence of branchial clefts III and IV and branchial arches III and IV, the greatest contribution being from the region posterior to cleft III (Fig. 32). The ductus branchialis III forms a small diverticulum of the cervical vesicle which becomes erased with extension of the vesicle (Fig. 34). In the sheep it can therefore be seen that the cervical vesicle IV originates from the placodal ectoderm posterior to cleft III but that cleft III and its closing membrane contribute but a small portion of the cervical vesicle. Closure of the ductus cervico-branchialis IV was seen to take place at 26 days (Fig. 7), (12·5 mm stage according to Batten, 1957). After its rupture soon thereafter, the cervical vesicle has an elongated globular shape. At 25 days it forms a pointed end between nodose ganglion and the anterior laryngeal branch of the vagus. In an embryo of 26 days (Fig. 32) it can be seen as an oblong, antero-posteriorly directed, epithelium-lined vesicle attached to the lateral part of the ganglion nodosum and lying mostly between it and the caudal part of the parathyroid. It is still connected laterally to the surface ectoderm by a cord of ectodermal cells (Fig. 7). Ventro-medially it is attached to the dorsocranial end of the thymus, i.e. by means of the persistent pharyngeal membrane III. A similar position is described for the rat (Rogers, 1929). The parathyroid is attached to the ventrocranial end of the thymus. This attachment in slightly older embryos becomes drawn out (due to caudad migration of the thymus) to form the parathyroid-thymic cord. This cord becomes absorbed again once the cervical thymus starts proliferating, giving the final position of the parathyroid III as shown in Fig. 28.

The cervical vesicle maintains an intimate contact with the nodose ganglion for a few days (to $\pm$34 days of age) (Fig. 10, 30, 33, 35) sometimes appearing partially within it but gradually separating therefrom while maintaining its contact with the dorsocranial end of the thymus. In older embryos it tends to move from the lateral to a medial position.

The pharyngeal membrane III is first seen to be ruptured in an embryo of 29 days (Fig. 35), the lumen of the cervical vesicle thereby becoming joined to that of the thymus tube. Already in an embryo of 24½ days cell debris is located in histiocytes in the vicinity of the pharyngeal membrane (Fig. 34). Mitotic figures are absent in it but numerous in the cervical vesicle itself. In the rat Rogers (1929) does not describe a rupture of the pharyngeal membrane but finds a decrease in size of the cervical vesicle due to a lessening of mitotic activity followed by eventual degeneration.

Throughout the development numerous mitotic figures are observed in both the cervical vesicle and the thymus indicating an active proliferation of both. No signs of degeneration in the cervical vesicle or thymus with the exception of that seen in the formation of corpuscles of Hassall (see later) are ever encountered embryologically.

In a transverse section through an embryo of 31 days old (Fig. 10) the position of the various structures is as follows: The parathyroid III is situated on the ventrolateral side of the cranial tip of the thymus cord; the cervical vesicle is attached to the most cranial tip of the thymus on the dorsomedial side of the parathyroid III; the internal carotid lies ventrally to the parathyroid III, the vagus dorsally and the jugular vein dorso-laterally. Caudally the thymus cord with its cordlike proliferations becomes pushed laterally by the contact of the internal carotid with the dorsal aorta. Beyond this contact the thymus cord moves medially, resulting in fusion of right and left cords in the caudal region just cranial to the heart.

The thymus at this stage consists of definite regions—there is the cervical portion followed by an intermediary narrower part (the intermediary cord of Badertscher, 1915 a). The cervico-thoracic cord joins the thoracic part, situated in the mediastinum, to the intermediary cord attached to the cervical thymus (Fig. 28). In slightly older embryos the cervico-thoracic cord may become severed on the right, the left or on both sides. Of these regions the cervical and thoracic portions show the most cordlike proliferations. These proliferations tend to be solid cords of cells whereas the primary downgrowth (Fig. 36, 37) shows a distinct lumen which at later stages only becomes obliterated.

Lymphocytes (=thymocytes) first appear at approximately 34 days of age and are, to all appearances, formed from the surrounding mesenchymal cells which become spherical and show numerous mitoses.

This phenomenon is first seen in the cervical region where, at this age, it is also most obvious. In the thymic cords of both cervical and thoracic regions occasional lymphocytic cells are already present between the thymic cells. Lymphoblastic types, derived from mesenchymal cells show enlarged, usually multiple nucleoli and basophilic cytoplasm. On the other hand, similar lymphoblastic types are also visible amongst the entodermally derived thymic cells. This observation, therefore, indicates the extreme difficulty encountered in trying to define the origin of the lymphocytes merely from cytological evidence. The lymphocytes so formed are mostly seen around the basement membrane of the thymic cords and gradually invade and facilitate reticulation of the entodermal cells. Surrounding blood-vessels also contain lymphocytes but these are believed to be carried away—due to the fact that the thymus is probably the first and most important lymphocytopoietic area in the embryo (Parrott & East, 1962). Histiocytes containing cell debris are quite numerous in the lamellated mesenchyme surrounding the proliferating cell cords. Such histiocytes are always typical for lymphocytopoietic areas, e.g. lymph nodes (Maximov & Bloom, 1957).

The cervical vesicle, as a result of the proliferation of the cervical thymus, comes to be enclosed in the cranial end of the latter, where it can be seen to give rise to numerous, solid, proliferating cell cords forming eventually thymic lobules (Fig. 36, 37). The cords arise by a proliferation of the basal cells of the stratified

epithelium lining the vesicle and may at times include a short evagination of the lumen of the vesicle. The cells lining the vesicle are easily distinguished by their clear distal portions—similar to that of stratified squamous epithelium of an identical age. Tordoir (1935) was unable to interpret such early stages of keratinisation. He tried explaining them by staining for fat (with Sudan III) but naturally with negative results. He could not stain for glycogen due to technical difficulties. Due to this inability to interpret the nature of the lining cells of the cervical vesicle he, in the opinion of the author, made the following incorrect conclusion:— " Wat ook de functie, wat ook het lot moge zijn van deze cellen, voor de opvatting dat de vesiculae praecervicales (author's cervical vesicle) waaruit zij stammen in staat zijn thymus weefsel te vormen, pleit geen enkel argument. Daarvoor is hun bouw te zeer verschillend van die van de thymus zelf ". The entodermal part of the thymus cord does not at this stage show such clear lining cells and the junction is therefore distinct (Fig. 36, 37). In the guinea-pig Klapper (1946) found ectodermal and entodermal cells so intimately fused that a definite boundary was indistinguishable. This is not the case in the sheep.

The exact amount of thymic tissue contributed by the cervical vesicle is at present impossible to determine but from several sections studied, and especially one of an embryo of 44 days old, it appears to be considerable. Indications of a cortex and medulla (the usual histological configuration) are first noticed in an embryo of 47 days.

Remnants of the branchio-pharyngeal duct appear to be able to give rise to smaller accessory parathyroids as one such mass of cells was seen in an embryo of 36 days old. Parathyroid-thymic cords of 90-130 μ long are encountered in some embryos. In the older embryos this distance between thymus and parathyroid is diminished due to growth of the thymus. The caudal portion of the parathyroid therefore becomes surrounded by the cranial end of the cervical thymus (Fig. 28). It eventually comes to be situated in the cervical region just caudal to the carotid body and branching of the common carotid, i.e. caudal to the point where the occipital and external carotid arteries are formed—the external maxillary artery being absent in sheep (Sisson & Grossman, 4th ed. 1956, p. 721).

It has been found that the position of the parathyroid relative to the vagus, thymus and internal carotid may vary a bit between right and left sides and between different embryos. This slight variation in position seems to be of no significance.

Ramsay (1948) transplanted third pharyngeal pouches of 10 day mouse embryos to young mice (one month old) and found that after four weeks well developed thymus tissue and cysts lined by either ciliated columnar or stratified squamous epithelium were formed by the transplants. These tissues were not encapsulated or walled off by host tissues. He also found that transplants of thyroid material from older embryos did not grow well but were plainly encapsulated by connective tissue and were degenerating although well differentiated colloid-filled vesicles had appeared. These experimental studies therefore, indicate that the third pharyngeal pouch of the mouse receives thymus-forming determination very early (at 10 days gestation or earlier), that the ultimobranchial body (attached to third pouch in mice) has no intrinsic thyroid-forming tendencies (see also later) and that transplanted embryonic thymus material is well tolerated by the host tissues whereas thyroid gland is not.

(2) *Discussion*

The discussion on thymus development can be grouped as follows:—

(*a*) *Epithelio-mesenchymal relationships*

That such a relationship exists was assumed by Kingsbury (1936): "Thymus thus expresses fundamentally an unusual interrelationship in development of two tissues, epithelium and connective tissue". This was later confirmed by Auerbach (1961): "Development of the thymus was seen to depend on an interaction between mesenchyme and epithelium". He found (1960) that a layer of mesenchyme one to three cells thick was sufficient to assure morphogenesis of the thymus *in vitro* and showed "that mesenchyme from a variety of tissue sources served as inducers of lobulation and growth". He proved experimentally that neither the epithelial nor the mesenchymal component of thymus "can develop in isolation, *in vitro* or *in vivo,* but clustering of the two tissues leads to restoration of the morphogenetic system with resultant *in vitro* lobulation and the *in vivo* formation of lymphoidal tissue". Therefore, although such an epithelio-mesenchymal relationship can only be assumed to exist from a morphologic study of the development it must be (and has been) substantiated by experimental work such as that of Auerbach.

(*b*) *Origin of lymphocytes* (*= thymocytes*)

The question of the origin of the thymocytes is a longstanding one and has given rise to a considerable literature. In the series of sections studied it was found that the initial lymphocytes in the case of the sheep are, to all appearances, of mesodermal origin. They were seen to appear in the mesenchyme surrounding the thymic cell cords but occasional ones were seen to be present in the cords themselves. This supports Maximov's (1909) original views and the consensus of opinion of most authors (Maximov & Bloom, 1957) i.e. that of migration from without inwards. The possibility, however, that those within the cords could have originated from the entodermal thymic cells cannot be excluded on cytological grounds only.

In all lymphocytopoietic areas lymphocyte formation as well as destruction is encountered (Maximov & Bloom, 1957). Such phenomena are also seen in the connective tissue immediately surrounding the early thymic cords. It also occurs in the thymic cords themselves. In addition numerous mitotic figures are seen in mesenchymal cells, the cytoplasmic processes of which have mostly been withdrawn—thereby almost resembling wandering cells. These result in little groups of lymphocytes formed from a single mesenchymal cell. The lymphocytes invade and probably facilitate the reticulation of the entodermal cell cords (prior to invasion the entodermal cells become vacuolated).

According to McAlpine (1955) the first stage of reticulation of the entodermal cells of the thymus of the rat is also formation of vacuoles in the cytoplasm. The function of the entodermal cells, therefore, in the first place, appears to be the initiation of lymphocyte formation from the mesenchymal cells (or possibility of lymphocyte formation by proliferation also) and secondly their maintenance and continued proliferation during the foetal stage. After the initial formation of lymphocytes in the mesenchyme [in full agreement here with Badertscher's (1915 b), and Kingsbury's (1915 a) views] and their subsequent invasion into the thymic cords, their formation in the mesenchyme is stopped and further proliferation takes place mostly in the thymic cortex. The maximum formation of lymphocytes in the mesenchyme takes place between 40 and 44 days.

According to Badertscher (1915b) only large lymphocytes invade the thymic tissue and become smaller by repeated divisions. In the sheep large and small ones are seen migrating in the thymic tissue. As the lifespan of a lymphocyte is considered to be not more than 24 hours (Bessis, 1956) large numbers of these lymphocytes die off, become phagocytosed and are probably reconverted to metabolic substances—an important source of nucleic acids for the developing foetus (Krölling & Grau, 1960). This phagocytic activity is seen in the thymic tissue as well as in the surrounding mesenchyme. The entodermal cells each show a large nucleolus in a vesicular nucleus, indicating amongst others a possible role in ribonucleic acid metabolism. Mesodermal and entodermal reticular cells can be differentiated on the appearance of the nucleoli—usually single and large in the entodermal reticular cells and small and multiple in the mesodermal reticular cells. Lymphoblasts in the thymus and other lymphoid tissue on the other hand show one or two large nucleoli resembling, therefore, the entodermal reticular cells. Lymphoblasts in lymphoid tissue are usually derived from mesodermal reticular cells.

There is the question of proliferation of thymocytes within the thymic tissue. Do they proliferate from existing thymocytes, from mesodermal reticular cells or from entodermal cells? In the sheep no definite conclusion can be reached, as mitoses occur in all three types, being most numerous however, in the lymphoblasts (=thymoblasts). In later foetal stages mitoses in mesodermal and entodermal reticular cells are very difficult and sometimes virtually impossible to define. It appears therefore that in the foetus the normal supply of thymocytes is derived by homoplastic lymphopoiesis, i.e. from existing thymocytes (=lymphocytes) and lymphoblasts. As these lymphoblasts are large cells with round vesicular nuclei and distinct nucleoli staining generally darker than the thymic epithelial cells, it will cytologically be virtually impossible to state whether they are derived from entodermal or mesodermal reticular cells as both types can theoretically speaking, give rise to such morphological types. It therefore appears to be a problem to be approached by histochemical and experimental methods.

According to McAlpine (1955) high phosphatase activity of the entodermal cells is associated with the formation of lymphoblasts from them. His observations " are consistent with the view that the thymocytes are derived primarily from the thymic reticulum cells rather than from immigrant lymphocytes ".

Auerbach's (1961) studies demonstrate " that lymphoid tissue of the developing thymus originates in the epithelial component of the early thymic rudiment, the mesenchyme providing the initial inductive stimulus and serving to furnish stromal elements of the gland ". He has shown that thymic epithelium cultured alone is capable of forming lymphoid cells (provided that the thymus epithelium contained, as stated by him, no stromal cells. Such a view (the entodermal origin of thymocytes) does not satisfactorily explain the early presence of lymphocytes and mitoses of mesenchymal cells in the mesenchyme surrounding the early thymic cords. It will explain the presence of lymphocytes within the cords. Emigration of early lymphocytes will hardly be feasible with mitoses and phagocytosed cell debris in the mesenchyme surrounding the cell cords.

Parrott & East (1962) have shown that the thymus is important for the development of the immune response during embryonic and neonatal life. Lymphocytes (=thymocytes) are the cells held responsible for the formation of antibodies and

therefore their early embryonic formation is necessary. However, lymphocytes morphologically similar, need not necessarily have the same histochemical reactions in the cortex or medulla of the thymus or in different parts of the same organ (Smith, *et al.*, 1958).

New light has been thrown on the function of the thymus by Parrott & East (1962). They found that the thymus is probably also " responsible for initiating the production of lymphocytes in other lymphoid organs " and this initiation could be brought about by a cellular " seeding out process ". " Since the thymus is the main lymphoid tissue present in the foetal and new-born mouse and its removal within strict time limits at birth causes lymphopenia and lymphoid depletion of the spleen and lymph nodes, we conclude that it is the most important and probably the only source of lymphocytes during late embryonic and early neonatal life ". In view of this observation it can, therefore, be assumed that lymphocytes in bloodvessels around the embryonic thymus are actually being carried away from the thymus. Experimental proof therefore seems to favour an entodermal origin for the thymocytes.

(*c*) *Role of the cervical in thymus formation*

The cervical vesicle either participates in the formation of the thymus (e.g. pig, guinea-pig) or it degenerates, forming no part of the thymus (e.g. man, rat, ox).

In the sheep the cervical vesicle IV contributes to the formation of the thymus. This is in direct opposition to Tordoir's (1935) findings. He found the cervical vesicle still recognisable in embryos of 50 and 60 mm attached to or embedded in the epithelium of the rostral end of pouch III. Scothorne (1950), basing his views on theoretical and comparative grounds, believed that the cervical thymus may be partly of ectodermal origin. Prenant (1894) believed it to be only of entodermal origin.

The primary function of the cervical vesicle IV (primarily the vagal placodal ectoderm) is its contribution of ectodermal cells to the ganglion nodosum. Batten (1957) says: " The anchorage of the cervical vesicle to the nodose ganglion must be correlated with the fact that the placode is engaged in contributing cells to the ganglion during the final stages of closure of the sinus ". According to Batten (1957), this contribution takes place from the 19th to the 27th day of gestation. From the sections examined it can be seen that the attachment of the cervical vesicle to the pharyngeal membrane III is much more intimate than with the ganglion nodosum. This statement is clearly understood when it is borne in mind that the pharyngeal membrane represents the fusion of two basement membranes (of ecto- and entoderm), whereas contact with the ganglion nodosum is maintained, for a short period only, by migrating or transforming cells. A reflection of this statement is seen in sections showing distortions. In these the cervical vesicle usually gets torn from the ganglion but hardly ever from the pharyngeal membrane. This difference in degree of intimacy is therefore responsible for the fact that the cervical vesicle becomes enclosed in the head of the thymus.

Scothorne (1955) found that the entodermal component of the thymus contained glycogen from its earliest differentiation (12 mm) to the latest stage examined (340 mm). The vagal placodal ectoderm contained no glycogen but the cervical vesicle by 17 mm already showed small quantities of glycogen which increased further with incorporation in the head of the thymus. These observations of Scothorne, together with the author's findings of proliferating cell cords, give a fair degree of proof that the cervical vesicle actually becomes induced by the thymus

tissue to form similar tissue. It appears, however, that this induction is confined to the stratum basale, the more distally situated cells retaining keratinising abilities, i.e. having developed according to their prospective significance, or in other words, according to the normal fate they would have suffered, had they remained as ectoderm on the body surface. There they eventually would have developed into stratified squamous keratinising epithelium.

In the sheep it was found that the pharyngeal membrane III ruptured (Fig. 35). This was not observed in either the rat (Rogers, 1929) or in the guinea-pig (Klapper, (1946). In the sheep, mitotic activity was found to remain constant in the cervical vesicle and not to diminish as in the rat. These observations also point to participation of the cervical vesicle in thymus formation.

Is the formation of a reasonably large cervical thymus therefore due to proliferation mainly from the cervical vesicle, mainly from the entodermal component, or due to approximately equal contributions from both? Without experimental or even histochemical evidence, it would be impossible, in the author's opinion, to answer such a question unhesitatingly.

(*d*) *The significance of the Corpuscles of Hassall*

It is assumed that the thymic cords, originally a stratified epithelium with tendencies towards keratinisation (similar to the pharynx epithelium) retain these general properties notwithstanding the reticulation.

These properties become evident in the formation of the corpuscles of Hassall. Smith & Parkhurst's (1949) conclusion was " that a process of keratinization is going on in these bodies similar to that in thick skin ".

It is found that at the periphery of the lobules where the metabolic activitiy of the epithelial cells is highest (as in stratified epithelium) the lymphocytes are most numerous—reticular cells relatively few—whereas in the middle the proliferative activity must necessarily decrease resulting in fewer lymphocytes, a relatively larger amount of reticular connective tissue in relation to the epithelial cells and therefore a tendency towards their epithelial arrangement. [Epithelial cells in contact with mesenchyme become arranged in an epithelium or in absence of a free surface as a round mass or vesicle (Balinsky, 1960)]. These cells then assume the typical whorled appearance forming corpuscles of Hassall which in the larger corpuscles of Hassall may show keratinisation and cell debris (Fig. 39). Ladewig (cited by Smith & Parkhurst, 1949) concluded that skin and Hassall's corpuscles undergo similar changes. He stressed the fact that cornification should not be considered as a degenerative process.

In the rat, McAlpine (1955) found that in the early stages of development, the periphery of the thymus showed a single zone of increased phosphatase activity; in later stages two zones were shown, subcapsular and at the cortico-medullary junction. Increased phophatase activity seems to be associated with differentiation of the large lymphocyte. James (1956) found phosphatase activity at sites of lymphocytic infiltration in the tonsil. These observations therefore support the present author's opinion that proliferative activity of lymphocytes coincide with metabolic activity of entodermal epithelial cells.

During the later foetal stages of thymic development large, often elongated corpuscles of Hassall are found in the centre of the medulla. These are the remnants of the primary thymic cords which, due to partial keratinisation, had not become reticulated but instead were filled with desquamated cells, keratohyalin

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granules and debris of invading cells such as lymphocytes and neutrophils. These large corpuscles of Hassall may become broken up into smaller ones by a rounding off of the entodermal cells from the periphery or an active breaking up by enzymes of the invading neutrophils. In the centre of the medulla a mass of closely associated smaller corpuscles of Hassall may therefore also be encountered (Fig. 39).

Steiner (cited by Smith & Parkhurst, 1949), by implanting pellets of methylcholanthrene into thymuses of young guinea-pigs, caused hyperplasia of Hassall's corpuscles and surrounding epithelial reticular cells. The presence of tonofibrils and keratohyalin granules in these new cells was sufficient proof of their epithelial nature.

It appears possible that the cervical vesicle may play a role in the initiation towards partial keratinisation and the formation of corpuscles of Hassall. The reasons for this view may be summarised as follows: —

(*a*) Its clear cells with a tendency towards keratinisation are distinguishable at an early age from the unkeratinised cells of the rest of the thymic cords. These cells show dark nuclei and may easily be mistaken for degenerating instead of cornifying cells.

(*b*) Its lumen at ± 29 days is brought in contact with the rest of the lumen of the thymic cords by the rupture of the pharyngeal membrane III. It can be assumed that certain enzymes are responsible for cornification and this raises the possibility that these enzymes may spread through the fluid in the lumen and so restimulate the entodermal cells to cornify.

(*c*) The cervical thymus is the first part to completely undergo age involution. Is this in any way connected to the presence of the cervical vesicle?

Hassall's corpuscles are not believed to have any special function apart from those involved in their inherent nature as stratified squamous epithelium (Smith & Parkhurst, 1949). Ladewig (1947), however, raises the question whether the Hassall corpuscles—because of their keratin content—are not functionally involved in the cystine metabolism of the body.

The development of the thymus must be seen as a result of an early epithelio-mesenchymal interaction resulting in (*a*) proliferation of cell-cords from the primary ventrocaudal downgrowth of pouch III which has intrinsic thymus-forming potencies, and (*b*) formation of lymphocytes from mesenchyme (or possibly from thymic entodermal cells) and the subsequent invasion and proliferation of these in reticulated entodermal cells. That the entodermal cells from the pharyngeal region induce or attract lymphocytes, is clear as it can also be seen in tonsil formation (James, 1956), lympho-epitheliomata and even in transplants. Lymphocytes are also found subjacent to and migrating through the lining epithelium of the whole digestive and respiratory tracts.

It can thus be seen that the prospective potency of stratified squamous epithelium, whether ectodermal or entodermal, can be the same provided it is surrounded by the mesenchyme of the cervical region before any other specific determination has taken place.

V.—Development of Complex IV

(1) *General Observations*

At 18 days of age it can be seen that the foregut loses its triangular shape caudal to the third pouches and becomes compressed laterally. The anlage of complex IV is formed at 18 days of age (15 mm) as small lateral pockets cephalodorsal to the auricles. The primitive pericardial cavity, therefore, prevents an early lateral extension of complex IV. At 20 days (6 mm) complex IV has formed a caudally directed, blindly ending pouch which never makes contact with the ectoderm and which already shows a small ventral pocket—the ventral diverticulum or true pouch IV (Fig. 3, 4, 5). The heart has moved caudad and is in no way in close proximity to complex IV. The lumen of complex IV is large and just slightly smaller than that of the pharynx itself. It is lined by a stratified epithelium similar to that of the pharynx. Cephalodorsally the fourth aortic arch passes over to contact the dorsal aorta (Fig. 1).

Tordoir (1935) raises the question of a fifth aortic arch and believes such to be represented by a capillary which he found between the ventral diverticulum and the ultimobranchial body. He considers the ultimobranchial body therefore as a fifth pouch which shares a common branchio-pharyngeal duct with pouch IV (the ventral diverticulum). He therefore considers the triangular piece of tissue between the ultimobranchial body and the ventral diverticulum as a fifth branchial arch. He describes a small branch of the vagus which runs in the direction of the triangle but could not be followed all the way due to unsuitable staining methods. He says: " Vermoedelijk zou, bij een daartoe geschikte kleuring, dit bundeltjie mischien wel geheel te vervolgen zijn geweest tot in bedoeld gebied ". Notwithstanding these observations he preferred the term of caudal complex as introduced by Tandler in 1909.

As the presence or absence of a capillary representing a fifth aortic arch seemed to be of crucial importance it was thoroughly investigated.

A small capillary, arising from the base of the fourth aortic arch and connected to a rich capillary plexus on the lateral aspect of complex IV, was found to be present in sheep embryos of 21 days. At 24 days (Fig. 6) there were actually two capillaries on the left side whereas on the right side the second was growing out. The larger of the two in each case arose from the base of the fourth aortic arch and passed ventrally between the ventral diverticulum and the ultimobranchial body to connect up with the rich lateral capillary plexus. This plexus was further connected by several arterial capillaries to the sixth arch and by one to the fourth arch.

In an embryo of 21 days it was found that the sixth aortic arch, which forms the caudal constriction of complex IV, forms a rather peculiar delta-like contact with the dorsal aorta at the point where the fourth aortic arch joins the dorsal aorta (Fig. 1, 4, 5). The number of branches vary slightly in different individuals both as regards to number and size. A more or less typical representation is seen in Fig. 4. The most probable interpretation of this delta-like contact of the sixth arch and dorsal aorta would be that it represents the phylogenetic disappearance of the most caudal aortic arches. As such it represents a highly controversial point which, as it is beyond the scope of this paper, cannot be considered any further here.

The question now arises whether capillaries arising after the sixth aortic arch, but cranial to it could be considered as aortic arches. In the author's opinion definitely not. Developmentally the arches arise consecutively in a cranio-caudad sequence. At 21 days only one of these capillaries were formed (but delta-like contact of sixth aortic arch was best developed) whereas at 24 days they were still progressively being formed. At 24 days the delta-like contact had only one or two branches left, the others being absorbed in the enlargement of the vessels. The variability of these capillaries was commented on by Tordoir (1935): "Enkele onderzoekers wezen er op, dat het verloop van deze vijfde kieuw arterie zeer variabel is, en hoe zij in vele gevallen in de plaats van een scherp begrensd vat een vaatplexus vonden".

It appears therefore that the additional two capillaries and the lateral plexus of complex IV are formed merely to provide nutriments for further development of the ultimobranchial body and parathyroid IV. At 26 days, with the parathyroid IV already well developed, some of the capillary contacts (especially with the fourth arch) are seen to be considerably enlarged and supplying the parathyroid IV.

Due to the presence, therefore, of at least two capillaries which could possibly represent aortic arches it would be unwise to postulate the existence of only a fifth aortic arch. As no comparative support for more arches exists among mammals the author is hesitant for enumerating any more. It is therefore considered that the capillaries develop as a result of an induction by the formation of complex IV.

As regards a nerve bundle from the vagus between pouch IV and the ultimobranchial body, the author came up against the same difficulty as Tordoir (1935) namely that to follow a small nerve bundle special techniques are required and these are not available at present.

Parathyroid IV ["thyroid glandule" of Prenant, (1894)] is first seen at approximately 20 days of gestation as a thickening of the dorsolateral wall of complex IV where it is in close association with the ganglion nodosum. From the sixth and fourth aortic arches very distinct capillaries are given off to this thickening where they form a capillary plexus (as discussed above) (Fig. 4, 5 C.P.A., 6). Here we have relationships similar to that found with the anlage of parathyroid III—Vagus and its ganglion nodosum cephalodorsally, the sixth aortic arch mediodorsally and a capillary plexus dorso-laterally. Such an arrangement of ganglion nodosum, aortic arch, capillary plexus and pharyngeal pouch must, therefore, seemingly be present for induction of the parathyroid.

As further development of complex IV is closely incorporated with the development of the thyroid it will be further considered under that heading.

(2) *Observations on Thyroid Development*

The thyroid development in the Merino sheep follows the usual pattern as described for mammals in general (Arey, 1959). It originates between the fifteenth and sixteenth day after fertilisation as a ventro-caudal conical projection of the floor of the pharynx in the midline just anterior to the second pouch (Fig. 8) and just dorsal to the angle between the truncus arteriosus and the anlage of the auricles. The outgrowth forms a conical depression in the floor of the pharynx, the foramen caecum, which, with later development of the tongue, gradually disappears leaving no trace in the adult. No knoblike intrusion into the pharyngeal cavity is formed as has been described for humans (Politzer, 1956). It is suggested that the mesenchyme of the truncus arteriosus or auricles actually induces the formation of the thyroid. Willier & Rawles (1941, cited by Rudnick, 1952) suggested that the heart mesoderm plays a role in thyroid induction.

At 18 days of age the anlage is tubelike, ± 30 μ long, thickened distally and with a very narrow lumen. It lies in the midline just cephalodorsal to the truncus at the fork of the second pair of aortic arches (Fig. 1). The first aortic arch at this stage is much smaller than the remaining arches and has already lost its contact with the truncus arteriosus. Growth in length is fairly rapid and at 20 days has reached ± 180 μ in length with its long axis directed antero-posteriorly. Mesenchyme has condensed around it forming a basement membrane, the latter easily visible after Mallory Azan staining.

At 20 days 17·5 hours of age the thyroid anlage has reached 190 μ in length and has migrated to a position just ventro-cranial, but in close contact to the truncus arteriosus, its caudal end lying just ventral to the point of origin of the third pair of aortic arches (Fig. 1 T.). The foramen caecum is reasonably large and narrows down to a thin epithelium-lined duct—the thyroglossal duct (primary secretory duct of Prenant, 1894). Caudally the duct becomes narrowed to an even greater extent, but distally the structure as such bulges considerably due to the proliferation of the epithelium resulting in radiating and anastomosing cell cords. As a result of the epithelial proliferations the extreme caudal region does not contain a lumen at all. This proliferating anlage is surrounded by a rich capillary supply, which with the surrounding connective tissue becomes incorporated as the interstitial connective tissue of the thyroid, thus giving rise to its very rich vascular supply.

The thyroglossal duct ruptures at approximately 23 days of age (9 mm) resulting in a more compressed thyroid with only a very small lumen visible. In a 24-day embryo the proliferating thyroid shows considerable lateral expansion and an increase in connective tissue centrally. It is compressed antero-posteriorly and extends caudally just beyond the point of origin of the third and fourth aortic arches i.e. lying in the fork of these arches. It has a length of 110 μ. The caudal apex shows the commencement of the bilobed structure.

At 26 days of age the thyroid has lengthened to ± 250 μ and is still located in the fork of the third and fourth aortic arches. It is triangular in shape, with no lumen visible, compressed dorso-ventrally as a plate of cells with its lateral extremities just ventral to the third and fourth aortic arches (Fig. 27). These lateral extremities have pointed ends directed craniad. Caudally the thyroid has a median pointed end.

At 27 days of age it can be seen that the aortic arches have receded caudad from the thyroid, causing its lateral extremities to curve dorsally and more craniad. By so doing they actually curve round and come in intimate contact with the lateral, ventral and ventro-medial aspects of complex IV. Complex IV at this stage consists of parathyroid IV dorsally, pouch IV as a hollow protuberance ventrolaterally and the ultimobranchial body forming the main bulk of the complex and extending most caudad (Fig. 17, 29). Complex IV loses its contact with the pharynx usually at or just below the height of the arytenoid swellings, at approximately 26 days. Complex IV at this stage is much larger than the lateral lobes of the thyroid and has a mass of cells protruding into its lumen from the ventro-medial aspect causing the lumen to be C-shaped (Fig. 16). The lumen is mostly lined by tightly packed cells some of which tend to be low columnar. The caudal aspects of the ultimobranchial body become completely surrounded by the thyroid tissue (Fig. 18). Just caudal to the ultimobranchial bodies the isthmus of the thyroid is formed which extends caudally only on the ventral side of the trachea with the thymus cords just lateral to the ventral thyroid tissue. Thymus III and the thyroid never at any stage of the development come in contact with each other.

It is interesting to note that the caudal extremity of the ultimobranchial body usually presents two, sometimes three, separate pockets, the most ventro-lateral of which is the thymus IV diverticulum (= pouch IV) (Fig. 18, 29). Do the remaining two pockets represent a stage in the phylogeny of branchial pouches V and VI or are they simply the initial formation of branches of the ultimobranchial body which can in older foetuses be seen in the caudal regions? The latter is the most obvious interpretation although the former may be used in order to explain the size and the shape of the lumen of the ultimobranchial body especially in relation to that of the other pouches.

Cytologically, two cell types can be distinguished in the ultimobranchial body in embryos of ± 28 days old (compare Fig. 19). They are (*a*) the chief cells, composing the largest mass of the complex, tightly packed with ellipsoid nuclei and indistinct cytoplasm, and (*b*) columnar cells with slightly elongated, oval, distally-situated nuclei and proximally a clear cytoplasm. The latter cells are situated in small groups in the lining of the ultimo-branchial body on the lateral and dorso-medial aspects. It is suggested that these clear cells are the first indications of the clear cells noted by Van Dyke (1945) in the postnatal ultimobranchial body (see page 213).

In the earlier stages complex IV is formed by a definite anterior pouch IV and posterior ultimobranchial body connected to the pharynx by a common branchio-pharyngeal duct. At the age of 28 days the relative sizes of pouch IV and the ultimobranchial body have altered, leaving the pouch IV as a small pocket situated in association with the cellular mass of the ultimobranchial body mentioned above. The branchio-pharyngeal duct passes dorsomedially from the cranial end of the ultimobranchial body just dorsal to the anlage of the thyroid cartilage where it becomes severed. It leaves a short, blindly ending, caudally pointed portion of the branchio-pharyngeal duct attached to the lateral side of the pharynx, lateral to the arytenoid swellings.

It could be expected that this portion of the duct or remnants of it (at point of severance) could later on give rise to cysts in or near the dorsal part of the lateral wings of the thyroid cartilage.

Parathyroid IV is seen as a dorsal proliferation of the epithelium lining complex IV just at the cranial end of the lateral lobes of the thyroid and not surrounded by thyroid tissue at this stage.

With the incorporation of complex IV into the lateral lobes they develop rapidly into the typical elongated lateral lobes of the sheep thyroid. The isthmus, which is fairly large at the beginning, gradually thins out so that in older foetuses and in adults it is sometimes hardly visible. In this connection Tordoir (1935) states: "Veelal bestaat deze isthmus slechts uit bindweefsel". This period of rapid growth resulting in a thin or absent isthmus was also reported for the rat (Rogers, 1929) and for the bat (Selle, 1935).

At 36 days (33 mm) the ultimobranchial body which is almost completely surrounded by thyroid tissue shows advanced budding of cell cords which then tend to radiate between the thyroid cell cords (Fig. 20). The cords initially can be clearly differentiated as the thyroid cords are more eosinophilic. The presence of the basement membrane when stained up facilitates differentiation even further. The position is often found where groups of thyroid cells are completely surrounded by groups of cells from the ultimobranchial body and *vice versa.*

The relation can often be so intimate that identification becomes difficult, especially further away from the ultimobranchial body. Already, therefore, at this early stage is it found that the cells of the ultimobranchial body are assuming the staining properties of the thyroid cells—almost sufficient proof for the induction theory of Rogers (1927).

Scothorne (1955), however, found glycogen to be absent from the thyroid at all stages, but present in the ultimobranchial body from 17 to 60 mm ($\pm$26 to 45 days) stages. Thereafter with cyst formation glycogen is progressively reduced. In the latest stages, however, he also found follicles arising from the cyst wall and identical with those of the thyroid itself. Therefore, the initial difference in staining properties is apparently due to differences in their chemical nature.

No further development of thymus IV could be traced. Once incorporated into the thyroid, further development is completely suppressed and its lumen forms part of that of the ultimobranchial body. It probably participates in the formation of the cellular mass associated with the ultimobranchial body (Fig. 16). It is, however, not associated with any influx or formation of lymphocytes. To what extent its presence can influence the formation of the small lymphocytic infiltrations often seen in older thyroids, seems absolutely impossible to determine. No evidence of any definite thymus IV tissue was found at all—with the possible exception mentioned on page 212. In this respect the author's findings agree with those of Kingsbury (1936) for the calf: "No evidence is found supporting the interpretation of the 'Ventral diverticulum' of the fourth pharyngeal pouch as the anlage of thymus IV. No specific thymus-forming area in the branchial epithelium could be selected; nor could any specific cell type be found which determines the thymic transformation".

The comparative sizes of the lateral thyroid lobes and the ultimobranchial body have changed. The latter, which in younger embryos is much larger than the thyroid lobes, has become relatively smaller, i.e. either a suppressing influence of the thyroid on complex IV is already seen—or the ultimobranchial body has stimulated thyroid development. Without experimental evidence it is a difficult question to settle. The rate of growth of the thyroid must necessarily be higher than that of the ultimobranchial body, or else inclusion of complex IV would not be so complete. Prenant (1894) found mitotic figures to be rare in the "lateral thyroid" (=complex IV) but more numerous in the median thyroid. The rate of growth of the two lobes of the thyroid is not the same. At 40 days of age the right lobe extends further caudad than the left; the same applies to the cranial extent and size. These are considered to be individual variabilities. The lumen of complex IV also rounds off with an invagination caudally. The expansion of the lumen necessarily causes the surrounding thyroid cell cords to become compressed against the wall of the complex giving a radiating appearance. Individually the shape of the lumen may, however, vary considerably. It is lined by a single layer of cuboidal cells with clear distal portions. These clear distal parts of the cells of the ultimobranchial body facilitate identification of tubular structures derived therefrom. They were also noticed by Prenant (1894). A stratified appearance is often seen, due to budding and the close association of the thyroid cords. Mitotic figures are encountered in the lining epithelium and in the cell cords.

The overall shape of the ultimobranchial body at 44 days is as follows: Cranially and caudally there is a blindly ending elongated pouch flattened dorsoventrally with the cellular mass centrally. The lumen passes over this mass ventro-laterally. Dorsomedially to it the parathyroid can be seen closely attached. The whole structure is included approximately centrally in the lateral lobes with the general direction from mediodorsal to ventrolateral and ending caudally just before the isthmus is formed. The lateral thyroid lobes have also at this age assumed the typical adult shape.

Follicle and colloid formation were first observed in a foetus of 55 days of age (90 mm). As these follicles were obvious it can be safely stated that they first formed a few days earlier, coinciding, therefore, with the observations of Barnes, *et al.* (1957, 1958) that thyroid follicles were first observed histologically at 52 days of gestation (85 mm). They also demonstrated experimentally that the initial I^{131} uptake occurs on the 50th day of gestation, i.e. a few days prior to actual colloid production. Once intermingled, cell cords originating from the ultimo-branchial body are no longer distinguishable from those of the thyroid. Only at 58 days of gestation (105 mm) could follicles and, therefore, also colloid be identified in cell cords still clearly originating from the ultimobranchial body. These thyroids, however, showed hyperplasia, to which phenomenon can be attributed an unexpected early stimulation of follicle-forming abilities in the cell cords of ultimobranchial body origin (see also below). It, therefore, appears that follicle formation takes place first in the true thyroid follicles and somewhat later in cords derived from the ultimobranchial body. In the pig, colloid-containing follicles from the median thyroid anlage were first seen in the 75 mm stage and from the ultimobranchial body anlage only at the 125–145 mm stage (Badertscher, 1918). It is virtually impossible to determine how much of the thyroid tissue is formed from the ultimobranchial body. Histochemical methods may probably in future solve this problem.

The thyroids of the 58-day old foetus were slightly larger than could be expected and showed considerable hyperplasia (possibly due to iodine deficiency). The epithelium lining the ultimobranchial body was papilliferous whereas the left lobe showed a smaller medial and larger lateral vesicle. Around the latter, connective tissue was increased. Attached to the medial portion of the right parathyroid, a small cyst lined by stratified squamous epithelium was present, it being the first indication of cyst formation. In older foetuses with hyperplastic thyroids large " central canals " are seen.

In a foetus of 63 days (125 mm) a possible anlage of a thymus IV was encountered ventromedial to the left thyroid lobe at the site of entrance of the superior thyroid artery (Fig. 38). No definite proof of it being potential thymus tissue could, however, be found. It was connected by a thin cord to the medial part of the thyroid but was definitely not aberrant thyroid tissue because no follicles were seen and the capillary supply was much poorer than that of the thyroid. The thyroid also showed the usual small lymphocytic infiltrations, poorly developed connective tissue stroma and generally a high cellular activity. The lumen of the ultimobranchial body was filled with colloid and occasional desquamated cells. The lining epithelium of the ultimobranchial body usually appeared darker than the true thyroid epithelium, due to palisade arrangement of the cells (Fig. 20). That, however, does not apply to its proliferating cell cords. Kingsbury (1935 a) however, described a deeply chromatic character of the whole complex throughout its development, thus enabling him to differentiate it readily from thyroid tissue in successive stages of its incorporation.

At approximately 80 days (195 mm) it can be seen that the ultimobranchial body is in the form of a couple of small follicles with branching cell cords, all surrounded by an abundant amount of connective tissue and bloodvessels. Only the tips of the cell cords penetrate in between the thyroid follicles, which at this stage are very prominent (Fig. 22). Follicles formed from the ultimobranchial body also contain a small amount of colloid but the colloid is less eosinophilic than the rest. These follicles are limited to the central and hilus regions and actually merge with the rest of the thyroid with no definite or identifiable margin.

Clear and dark cells are distinguishable in the thyroid and ultimobranchial bodies. Van Dyke (1945) described the clear cells as originating from the ultimobranchial body.

The right parathyroid in this foetus was attached to the thyroid by means of a group of very irregular vesicles which could be followed through to the ultimobranchial tissue. The radiating cords arising from the latter reached the lobules where they ended in follicles with a sightly pink staining colloid. Thyroid follicles normally appeared rounded or oval, whereas those from the ultimobranchial tissue could be variously shaped and often showed budding (Fig. 21). Large ultimobranchial follicles could eventually disappear in a mass of smaller follicles. Such large follicles of ultimobranchial body origin, especially those near the parathyroid, had a lumen filled with an atypical, stringy or reticulated and lightly eosinophilic colloid, whereas true colloid is homogeneous and strongly eosinophilic (after Zenker's fixation). Occasionally, strongly basophilic mucoid masses were present in such large follicles (Fig. 23).

The cellular mass described previously as associated with the ultimobranchial body also gives rise to colloid-containing follicles.

In older foetuses it was seen that where large ultimobranchial follicles were present they invariably showed a part of the lining epithelium, especially that adjacent to connective tissue surrounding bloodvessels, to be a typical stratified squamous epithelium (Fig. 24). Such areas were often interrupted by cuboidal epithelium. Even from the proximal parts of the stratified squamous epithelium it was seen that cell cords were formed which gave rise to thyroid follicles (Fig. 25, 26). Follicle formation in the developing thyroid was seen to take place as follows:—

(*a*) By lumen formation in cell groups which became pinched off from cell cords—this was the only method of formation seen to take place in the median thyroid tissue.

(*b*) By the formation of a hollow protuberance, i.e. evagination from a large follicle and subsequent division (i.e. branching)—mainly seen in large ultimobranchial follicles (Fig. 21).

(*c*) By the formation of cell cords from the ultimobranchial tissue and subsequent formation of follicles from the cell cords (Fig. 25, 26). The ultimobranchial tissue, therefore, virtually acts as reserve material from which new follicles arise.

In a foetus of 87 days (225 mm) it was seen that the ultimobranchial body caudal to the central cellular mass gave rise to three main branches—lateral, dorsal and medial—and these gave rise to a considerable amount of thyroid tissue. In this foetus a small bunch of cells and small cysts surrounded by areolar connective tissue were encountered on the mediocranial extremity of the right lobe of the

thyroid (cf. Van Dyke, 1945 his Fig. 8). This remnant of the branchio-pharyngeal duct of complex IV is a fairly constant occurrence in sheep. Close to it, in the hilus, large follicles of ultimobranchial origin are usually a constant feature; in some, slight cellular connections are still seen giving a true concept of their origin, viz. from the most cranial part of the caudal pharyngeal complex after its severance from the pharynx by the rupture of the branchio-pharyngeal duct. This cranial tip of the complex is not included in the thyroid and is, therefore, separated and surrounded by connective tissue. The possibility that this tissue could give rise to an occasional thymus IV exists, because according to Kingsbury (1936) no reason exists for thymus IV tissue not to arise from the ultimobranchial body. This tissue, according to Van Dyke (1945), exhibits retrogressive characteristics. No such thymus IV tissue was, however, encountered in the sheep.

The gradual development of epithelial cysts in the thyroid is seen in foetuses from approximately 80 days old (195 mm) (Fig. 25). The areas lined by stratified squamous epithelium in the large follicles mentioned above tend to form protuberances and these, by a " pinching-off ", are separated and become completely surrounded by fair amounts of connective tissue. Such cysts are, however, still able to give rise to cell cords and follicles in the late foetal stages. Alternatively, follicles are formed partly lined by stratified squamous epithelium and partly by cuboidal epithelium—the latter cells only forming colloid and not the former (Fig. 24). It appears that with the gradual increase of connective tissue and a diminishing of the capillary supply around the follicle, the cuboidal cells become transformed, giving rise to stratified squamous epithelium. The latter retains its activity and forms keratinised cells distally, gradually filling up the lumen of the follicle with compressed eosinophilic squames—thus forming an epithelial cyst. Simultaneously cell cords are formed proximally (Fig. 25), eventually giving rise to the typical appearance seen in a normal 6-tooth adult sheep (Fig. 26).

From sections examined it is distinctly clear that the ultimobranchial tissue can be recognised in most older foetal thyroid sections by the presence of—

(*a*) large irregular colloid-filled follicles, mostly centrally situated in relation to larger blood vessels and showing origin of new follicles by budding or from radiating cords (Fig. 21, 22);

(*b*) the lining epithelium of the larger follicles tending to be darker under low power due to closer arrangement of the cells (Fig. 21); and

(*c*) newly formed smaller follicles in later stages tending to show colloid which is less eosinophilic than the rest (Zenker fixed material) (Fig. 22).

(3) *Discussion on Complex IV development*

Despite considerable literature on the role of the ultimobranchial body and pouch IV respectively in thyroid and thymus development, much still remains to be said before a unanimous opinion can be given. This is primarily due to differences in interpretation, species differences and to the scarcity and extreme difficulty of obtaining the necessary experimental evidence as regards to mammals. However, histochemical evidence, still a reasonably new field, may in the near future help considerably. Kingsbury (1914) stated: " Despite careful study, I have been unable to satisfy myself as to the actual fate of the material so included in the medial portion of the lateral lobes of the thyroid ".

The difficulty which faces the various investigators as to the interpretation of results seen is further amply illustrated by Kingsbury (1935 a) as regards to thyroid development in man: if " the main and central mass of the inclusion undergoes loosening, reticulation and apparently ultimate degeneration, it by no means follows that the denser superficial layer of the ultimobranchial body may not undergo transformation to thyroid parenchyma ". He could not disprove it, but considers it " highly unlikely although possible ". Prenant (1894) found an uncertainty as regards to the exact nature of the contribution of the " lateral thyroid " (=complex IV) to the median thyroid, an uncertainty which he considered the most attentive examination would not dispel.

Van Dyke (1945) has shown, by means of a summary, the variability that exists in the alleged fate of the ultimobranchial body in the different mammals. It is a regressive structure in man (Kingsbury, 1935 b; Van Dyke, 1940 a) and in the cat (Mason, 1931). It participates in the formation of the thyroid parenchyma in the calf (Anderson, 1922), in the dog (Godwin, 1937 a), in the rat (Rogers, 1927) and in the pig (Badertscher, 1918, 1919). In lower vertebrates the ultimobranchial bodies are not connected with thyroid formation at all. In this respect Sehe (1960) states: " The ultimobranchial body of lower vertebrates never comes in direct contact with the thyroid gland nor does it even transform into accessory thyroid tissue ". Sehe also found no storage of iodine in the ultimobranchial bodies of lower vertebrates under conditions which gave high accumulation in the thyroid gland. He further suggests that they are " not of any endocrine importance ".

Comparatively speaking, the ultimobranchial body, therefore, has no intrinsic thyroid-forming potencies. Such is also the conclusion of Godwin (1940) for the pig. Kingsbury (1939) concludes that the term ultimobranchial body is merely descriptive without any implications as to a specific organ anlage. Formation of thyroid follicles from ultimobranchial tissue in some mammals can be based on induction by the thyroid tissue as originally postulated by Godwin (1939) for the dog. Consequently it is found that follicle formation from the ultimobranchial body takes place much later than from true thyroid tissue (see above).

The consensus of opinion as regards to pouch IV (ventral diverticulum) of complex IV is that it is responsible for formation of thymus IV. The subject has been comparatively reviewed for the pig, rat, cat, dog, calf and man by Godwin (1940) and repetition is unnecessary. Its occurrence, with possible exceptions of the cat and the opossum, is very variable and if present usually occurs late in foetal development. As regards to pouch IV in the pig Godwin (1940) states: " The extreme variability in development and even the absence of a well-defined fourth pouch in the pig seems to indicate that the fourth pharyngeal pouch is being lost." In the sheep the author found the position to be almost similar—a fourth pouch is formed in the sheep but it is completely obliterated once it is enclosed together with the ultimobranchial body in the thyroid. Complex IV in the sheep virtually, therefore, consists of a parathyroid IV and the ultimobranchial body—pouch IV being rudimentary although always present.

In the author's opinion it can be assumed that the ultimobranchial body and attached pouch IV would develop into either epithelial cysts or thymus tissue, provided it is surrounded by mesenchyme only and kept away from the inducing

influence of the thyroid. As no experimental evidence to this effect was encountered in the literature this assumption is based on the following observations:—

(*a*) Thymus IV tissue is formed, but not constantly, in the late foetal development as described by Kingsbury (1936) and Anderson (1922) for the calf, by Klapper (1946) for the guinea pig and by Van Dyke (1940 b) in the human. This thymus IV tissue is formed from the ultimobranchial body or pouch IV, usually in the vicinity of the parathyroid IV, where portions of the ultimobranchial body or branchiopharyngeal duct escape inclusion in the thyroid and become surrounded by areolar connective tissue (Fig. 38). This connective tissue then, is believed to stimulate the original tendencies to completion, viz. cyst or thymus IV formation. Such cysts have been encountered in the sheep whereas Groschuff has described the presence of an occasional thymus IV in the sheep (cited by Van Dyke, 1945). Kingsbury (1915 a) in this respect states: "It is altogether probable that the thymus transformation may befall any portion of the epithelium of complex IV which may persist in the mesenchyme."

(*b*) In the opossum a large portion of the mature thymus is constantly derived from the fourth pharyngeal pouch as described by Kingsbury (1940). Kingsbury also found that in the opossum the ultimobranchial body and pouch IV are separated, that pouch IV could be clearly followed, and that thymus IV was actually larger than thymus III.

(*c*) In the cat the ultimobranchial body and thymus IV, together, form the internal thymic lobule. The former two have the same prospective potency, viz. that of thymic transformation. "We have no reason to consider the potencies of the ventral diverticulum as any different from those of the ultimobranchial body since in the dog and rabbit as well as in the cat, both components of complex IV suffer the same fate" (Mason, 1931). She made a significant statement: "As a rule the more external (in its relation to the thyroid) the thymus is, the larger it is"—i.e. recession from thyroid influence in the author's opinion could stimulate original tendencies especially through reinstigation of mesoderm induction, or would it be due to a slower or incomplete initial incorporation into thyroid tissue and a longer subjection to mesenchyme induction? In the latter case it could be said that the original mesoderm induction had been more complete.

Grobstein (1953) was able to show through cultures of the rudiment of the submaxillary gland of the mouse that the mesenchyme of the submaxillary region was responsible for the differentiation of the acini and ducts. The inductive power could also be exercised across membranes of various types. If such lobule formation could be the influence of the mesenchyme on a neighbouring organ it is only logical to expect that the mesenchyme of the branchial region would at least, to a certain extent, control differentiation of the specific regions. Hilfer (1962) found that when dissociated chick thyroid cells have spread out in culture, "they seem to require thyroid mesodermal cells for re-establishment of follicles". Auerbach (1960) has shown a similar relationship to exist for the thymus. Balinsky (1960), in relation to transplantations of entoderm into ecto-mesodermal shells (gastrula stages) states: "Again we find that the entoderm, as well as the ectoderm is dependent on the mesoderm in its differentiation".

These observations, therefore, point to the fact that considerable importance must be attributed to the mesenchyme in the differentiation of the entodermal derivatives, thyroid, thymus and ultimobranchial body.

The thyroid primordium induced by heart or aortic sac mesoderm (see earlier) is normally and completely differentiated by contact with this or surrounding mesoderm which then becomes incorporated as interstitial connective tissue aiding further differentiation as needed. However, due to its lateral expansion (caused probably by growth tensions in turn caused by enlarging pericardial sac), it comes in the way of the complex IV which then necessarily must grow down into the thyroid. Prior to this inclusion complex IV had been in contact with mesoderm, but for a brief period only (correlations between length of this period and ultimate fate of complex IV would be very instructive but is beyond the scope of this paper). This period in the sheep has presumably been long enough to permit formation of a ventral diverticulum (Pouch IV) which subsequently becomes obliterated once it is enclosed by the thyroid. This is an example of incomplete induction (of ventral diverticulum) and subsequent suppression by a different and probably stronger inducing influence (that of the thyroid).

Balinsky (1960) states that " the morphological and physiological peculiarities of tissues require for their maintenance the environment which surrounds them in the normal organism. Except for manifestly non-living parts, such as the chitin cuticle in insects or the hairs in mammals, all other animal structures may become changed, or may even dissolve and disintegrate if the normal conditions in the organism are changed ".

Hilfer (1962) in this respect states: " Most current definitions imply that structural characteristics of a cell type are superficial and can change with alteration of the cellular environment. In spite of such structural changes each cell type once differentiated, is assumed to be able to return, under suitable conditions, only to that type of differentiation which had been exhibited previously ".

With the above in mind and the fact that the ultimobranchial body according to the consensus of opinion of various investigators is considered phylogenetically as a regressive structure with no organ-forming potentialities, its variability as regards to thyroid contribution, thymus or cyst formation in mammals is not surprising.

The thyroid parenchyma with its mesenchyme, therefore, virtually suppresses what could be said to be the normal developmental tendencies of the ultimobranchial tissue. This suppression could only be effected because the ultimobranchial body and ventral diverticulum at the time of inclusion are virtually still in an undifferentiated state—undifferentiated as regards to any glandular state but differentiated as regards to cuboidal or stratified epithelium.

The prospective significance of complex IV tissue is:—

(*a*) The complete differentiation of parathyroid IV tissue from its dorsolateral aspect which normally takes place before incorporation. Thyroid induction does not seem to effect completely differentiated tissue, such as the parathyroid, very drastically. It appears to be merely maintained, presumably in a static state, while the thyroid itself is relatively increased in size. It is significant, however, that parathyroid IV is often absent or represented only by a small number of cells in the thyroid of older sheep, giving the impression of a gradual but certain suppression of parathyroid IV morphology and function.

In connection with the parathyroid IV in the pig and opossum Godwin (1943) states: "It seems probable that the parathyroid IV starts to develop but later degenerates in both the opossum and the pig."

(*b*) Formation of occasional thymus IV tissue which phylogenetically should arise from pouch IV but in practice (none encountered by author) most probably (as discussed above) from remnants of complex IV not included in thyroid. From such unincluded tissue cysts may also arise.

Due to thyroid induction in the sheep the prospective significance is effected only as far as formation of a parathyroid IV is concerned. In the case of the ultimobranchial body and pouch IV it actually is the prospective potency which comes into effect and we first find formation of thyroid follicles from the ultimobranchial body after which it gradually is surrounded by varying quantities of connective tissue with formation of epithelial cysts.

Once these epithelial cysts are formed they tend to increase in size due to continued proliferation of epithelial cells into the lumen which, having no outlet, must increase in size (Fig. 26). As a result of this increase proliferating cords, follicles and connective tissue become compressed peripherally. The connective tissue becomes relatively decreased, forming a fibrous capsule which at this stage has merely isolating functions. The stratified squamous epithelium of the cysts, being fully differentiated, can, however, under certain abnormal states give rise to cell cords forming the so-called "new growths" of Van Dyke (1945). He has also shown (1942, 1944 a, 1945, 1950) that such cysts can completely transform into thyroid follicles if sheep are maintained on an adequate diet. Cutaneous application of methylcholanthrene in mice or feeding vitamin A deficient diet to young rats may cause metaplastic development of large epithelial cysts from thyroid-like vesicles contributed originally by ultimobranchial tissue (Van Dyke, 1948 abstract). Vitamin A deficiency and action of carcinogens, therefore, stress the tendency towards cysts and cyst-adenomata formation. In choline deficiency he found that these metaplastic structures may revert back to more primitive mucus-like epithelia characteristic of unincorporated ultimobranchial bodies (Van Dyke 1944 a, b, c).

The stimulation of these cysts, therefore, appears to be intimately associated with some aspects of metabolism of the individual as well as with the cellular environment. Whether metabolism directly affects the epithelium, whether this effect is conveyed through an increase of connective tissue or whether connective tissue increase or cyst formation are essentially different aspects of malfunctioning metabolism (or partially due to aging) are questions which will shed considerable light on this subject but cannot be answered at this stage.

Whether the formation of stratified squamous epithelium is actually induced by the connective tissue, i.e. a changed cellular environment, or whether it is merely a lack of induction and a reversion back to the original tendency of the pharyngeal epithelium, viz. the formation of stratified squamous epithelium, remains a controversial issue. It is significant that where such epithelium is formed it is always separated from the thyroid follicles by a fair amount of connective tissue. Such a reversion hypothesis appears to be quite acceptable, as it would simultaneously explain the occurrence of cysts lined by columnar ciliated epithelium and often containing mucoid substances (Fig. 23). Such cysts are

mostly encountered in or near the parathyroids (Thomas, 1930). However, the latter glands arise from the dorsolateral portions of complex IV which in turn arose more from the dorsal region of the pharynx i.e. from a region having tendencies for the formation of pseudostratified columnar ciliated epithelium with goblet cells. It can therefore be reasoned that in the case of cysts arising from such dorsal regions (i.e. from vestiges of the parathyroid-ultimobranchial body cord) such cysts will have columnar ciliated epithelium with or without goblet cells.

With the above in mind it can, therefore, be safely stated that the epithelial cysts do not develop as a result of vestiges of the thyroglossal duct, as advocated by Thomas (1930), but as a result of transformations of ultimobranchial tissue. As the complex IV becomes included in the lateral lobes of the thyroid on its craniomedial aspect (Fig. 17, 29), i.e. at the hilus, it is natural to expect that the hilus or vicinity would be the site for the localisation of most of the cysts.

Most of the smaller follicles derived from the ultimobranchial body were indistinguishable from the thyroid follicles and were not separated from thyroid follicles by a connective tissue sheath—merely by fine reticular fibres and capillaries. The author, therefore, cannot agree with Verdun in his statement concerning derivatives of the ultimobranchial body as being " a special glandular mass which is clearly separated from thyroid parenchyma by a connective tissue shell " (cited by Van Dyke, 1945). This connective tissue sheath actually develops around the cysts and to some extent probably causes them in later foetal life (as previously discussed), mostly forming a separation between the newly proliferating cell cords and those cell cords which at an earlier age have mingled with the thyroid tissue and formed thyroid follicles.

The conclusion reached by Verdun and Simon " that no real fusion occurs between the lateral thyroid and the median anlage in sheep and that their subsequent histories were independent " (cited by Van Dyke, 1945) is, therefore, also not applicable in this work.

Van Dyke (1945) in his statement, " Since it (ultimobranchial body) is frequently separated from thyroid parenchyma by abundant connective tissue, it might be regarded as lying morphologically outside the thyroid gland " had probably not taken into consideration the fact that the ultimobranchial follicles in the late foetal stage are formed prior to the formation of abundant connective tissue and that these follicles mingle with the true thyroid follicles. Only vestiges of the ultimobranchial body, therefore, remain surrounded by the abundant connective tissue characteristic for the sheep thyroid.

Due to the aberrant nature of the ultimobranchial body in the thyroid it seems plausible to suggest that it would primarily be effected by various pathologic and metabolic states. Such effects are especially seen in neoplasia (Van Dyke, 1945).

The conclusion is, therefore, reached that in the sheep the ultimobranchial body under the inducing influence of the thyroid gives rise by budding or proliferating cell cords to follicles indistinguishable from true thyroid follicles. These follicles become separated from the main body of the ultimobranchial tissue by connective tissue, causing thereby formation of epithelial cysts from the main body which, under suitable conditions, can further give rise to thyroid follicles only by proliferating cell cords. Pathologic processes and metabolic states appear first to affect such ultimobranchially derived follicles due to their inherent unstable nature.

VI.—Conclusions

The following conclusions have been reached in the study of the pharyngeal development of the sheep foetus:—

(1) Four pharyngeal pouches are formed, of which only the first three reach the ectoderm. The ultimobranchial body is attached to the vestigial fourth pouch and this structure together with the parathyroid IV anlage is considered as complex IV. Pouch IV loses its identity as complex IV becomes enclosed in the thyroid. No substantial proof was found for considering the ultimobranchial body as a fifth pharyngeal pouch.

(2) Pouch II is obliterated and leaves no derivatives. The palatine tonsil is formed secondarily at site of obliteration and region just anterior to it. A vestigial ventral diverticulum of pouch II (= thymus II) is formed but regresses completely within two to three days after formation.

(3) Pouch III forms the classical derivatives, parathyroid III and thymus III. Thymocytes are considered to be primarily of mesodermal origin. They migrate into the thymus where subsequent proliferation takes place from existing thymocytes or from mesodermal or entodermal reticular cells. No differentiation was possible between the latter two.

(4) Although complex IV does not reach the ectoderm a slight branchial cleft IV is formed. Cervical vesicles II and IV are formed; the former is completely obliterated whereas the latter contributes to the cervical thymus. Consideration is given to the possible role played by the cervical vesicle IV in the initiation towards partial keratinisation and the formation of corpuscles of Hassall.

(5) Parathyroid III and IV are formed, the former being incorporated into the head of the thymus whereas the latter becomes enclosed cranio-medially in the thyroid.

(6) The ultimobranchial body contributes to the lateral lobes of the median thyroid. Vestiges of the ultimobranchial body remain as cysts. It has, however, no inherent thyroid-forming potencies—these develop merely as a result of the inducing influence of the thyroid.

(7) The role of the mesenchyme is considered in thyroid and thymus development. It is probably responsible for lobe formation and its presence is essential for differentiation of the entoderm.

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ABBREVIATIONS USED IN PHOTOGRAPHS.

Anterior laryngeal nerve	A.L.n.
Aortic arches	AA, 1–6.
Arytenoid swellings	A.S.
Branchial arches	Ba. 1–4.
Branchial clefts	1–4.
Branchial duct	Bd. 2–4.
Branchio-pharyngeal membrane (closing plate)	M. 1–4.
Branchio-pharyngeal duct	BpD. 2–4.
Capillary to parathyroid anlage	C.P.A.
Carotid (internal)	C.
Caudolateral pocket	Clp.
Cervical vesicle	C.V. II, or IV.
Dorsal aorta	D.A.
Fossa tonsillaris anterior	F.a.
Fossa tonsillaris posterior	F.p.
Ganglion nodosum	Gn.
Glosspharyngeal nerve	N IX.
Hypoglossal nerve	Hn.
Jugularis	J.
Lung bud	L.B.
Mandibular process	MnP.
Maxilla	MxP.
Meckel's cartilage	Me.
Notochord	N.
Oesophagus	O.
Palatine fold	Pal.
Parathyroid	P.t.
Pharyngeal pouches	P.P. 1–4.
Pharynx	P.
Precardinal vein	PV.
Rathkes pouch	R.
Reichert's cartillage	Rc.
Sympathetic cord	S.
Thymus	Tm.
Thymus III anlage	T.A.
Thyroid	T.
Trachea	Tr.
Ultimobranchial body	U.
Vagus	V.
Vagal placodal ectoderm	Vpe
Ventral diverticulum	Vd.

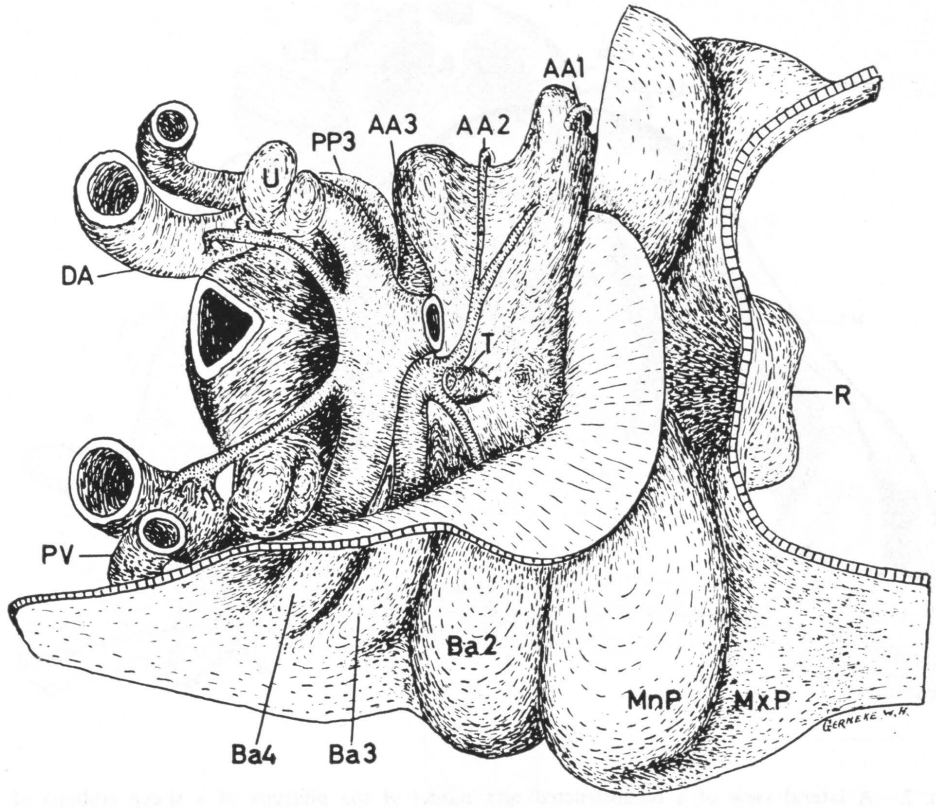

FIG. 1.—A ventral view of a reconstructed wax model of the pharynx of a sheep embryo of 20 days 17·5 hours. On the right side the relation of the aortic arches to pharyngeal pouches is seen whereas on the left side branchial arches are shown. Aortic arch I is shown as partially regressed. × 100.

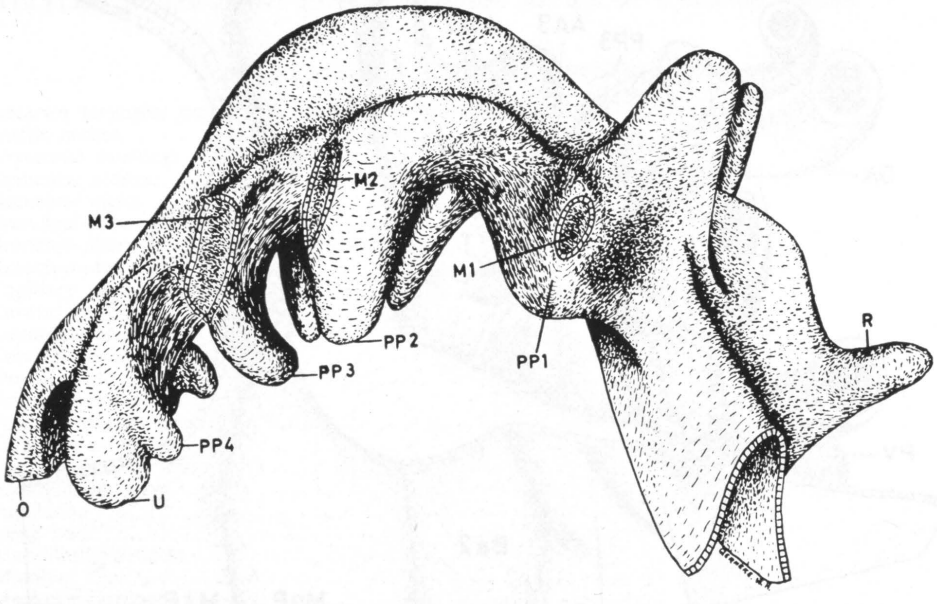

FIG. 2.—A lateral view of a reconstructed wax model of the pharynx of a sheep embryo of 23 days 23·5 hours showing branchio-pharyngeal membranes, Rathkes pouch and the anlage of the tympanic cavity, pouch IV and the ultimobranchial body. × 100.

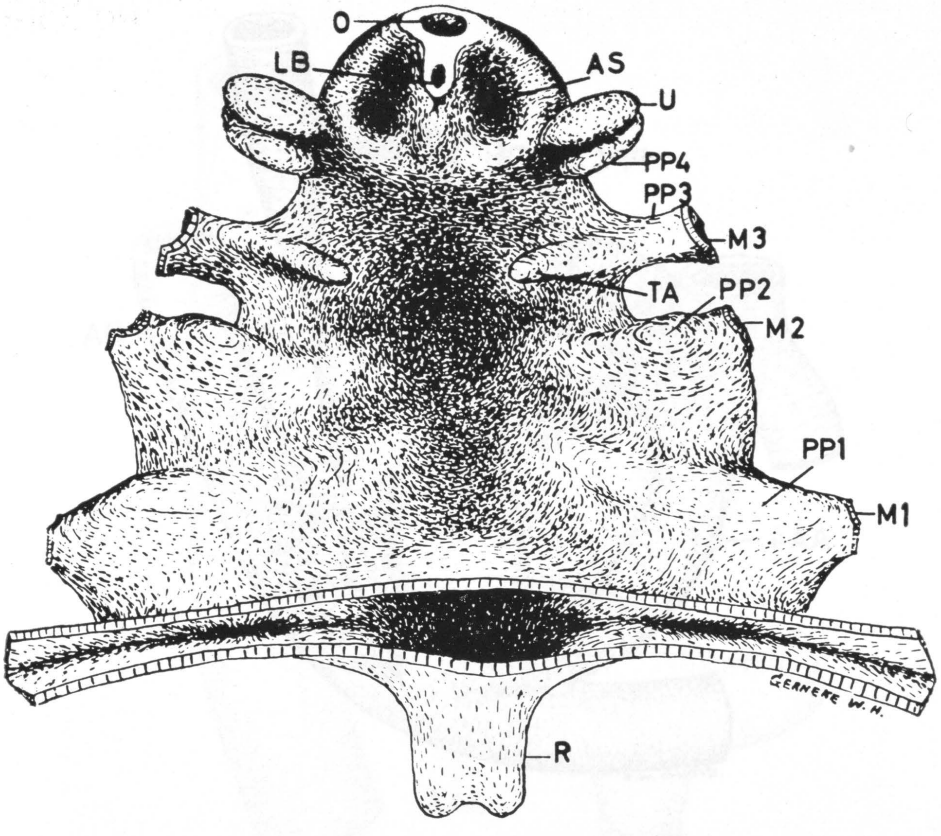

FIG. 3.—A ventral view of the same model as in Fig. 2.

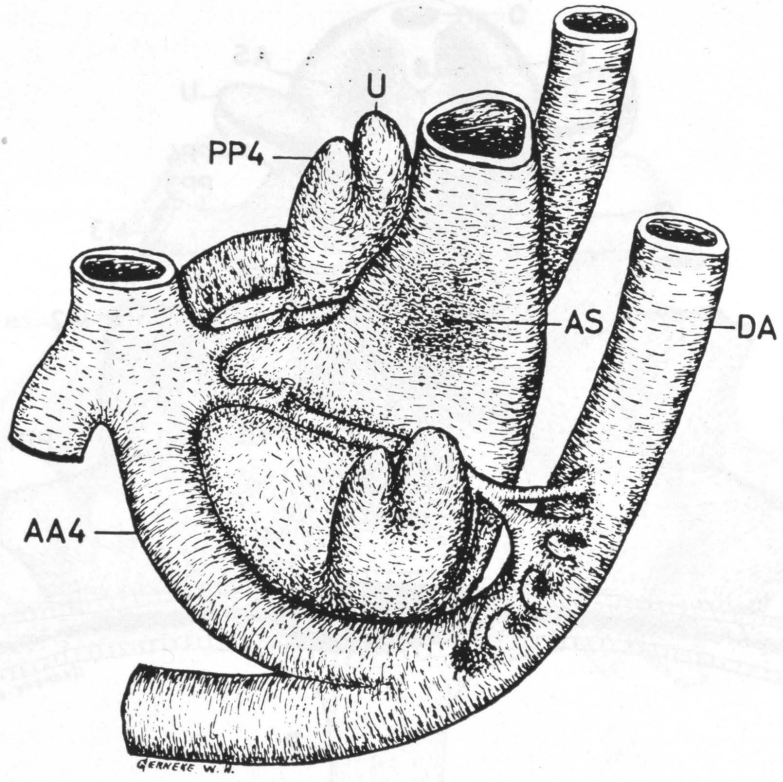

FIG. 4.—A schematic diagram showing the delta-like junction of the sixth aortic arch with the dorsal aorta of an embryo of 21 days—right side viewed laterally. The lateral capillary plexus (see Fig. 6) was omitted.

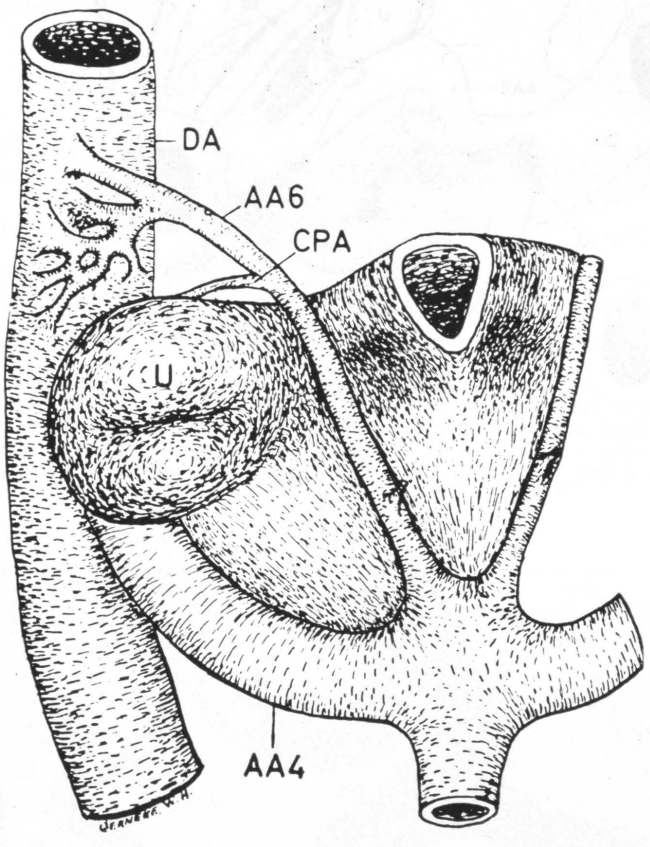

FIG. 5.—Similar to previous schematic diagram but showing the left side viewed ventrally. The lateral capillary plexus (see Fig. 6) was omitted.

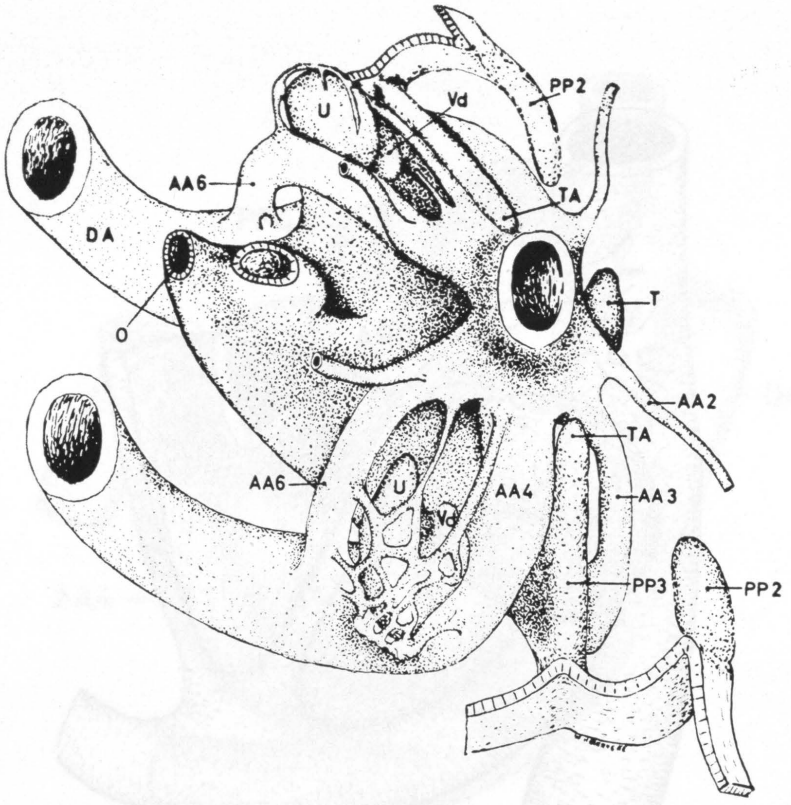

FIG. 6.—A ventro-lateral view of a reconstructed wax model of a portion of the pharynx and aortic arches of a sheep embryo of 23 days 23·5 hours. The lateral capillary plexus, its connections to aortic arches 4 and 6 and the two additional capillaries which could possibly represent additional aortic arches, are well shown. × 100.

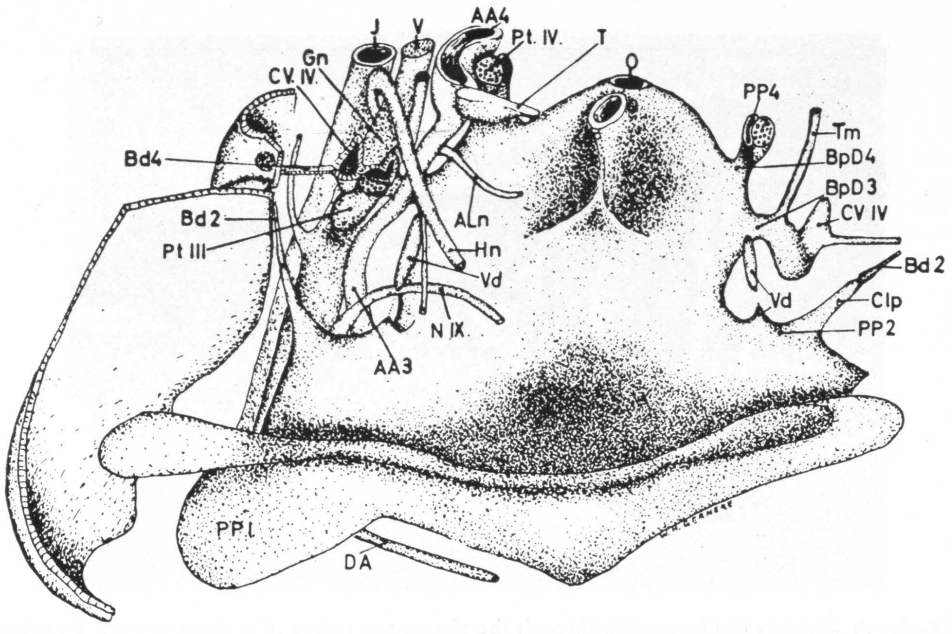

FIG. 7.—An antero-ventral view of a reconstructed wax model of the pharynx and the more important associated bloodvessels and nerves (left side only) of a sheep embryo of 26 days. Fig. 14 and 32 are transverse sections of this embryo at different heights. The relations of pouches 2, 3 and 4 and some of their derivatives to the pharynx are shown on the right side. × 50.

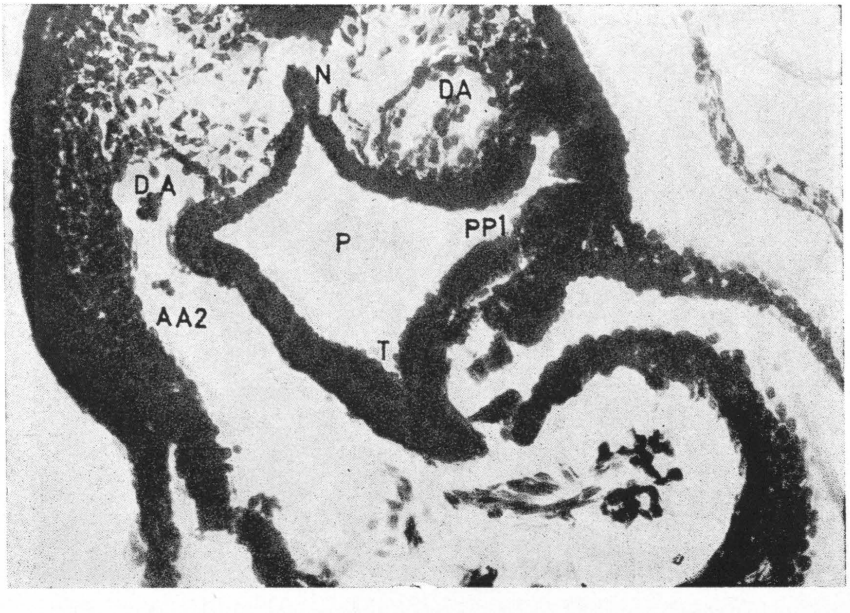

FIG. 8.—A photograph of the anlage of the thyroid (T). On the left side pouch I has already been partially constricted by the aortic arch II, situated just caudal to it (17 days). × 192.

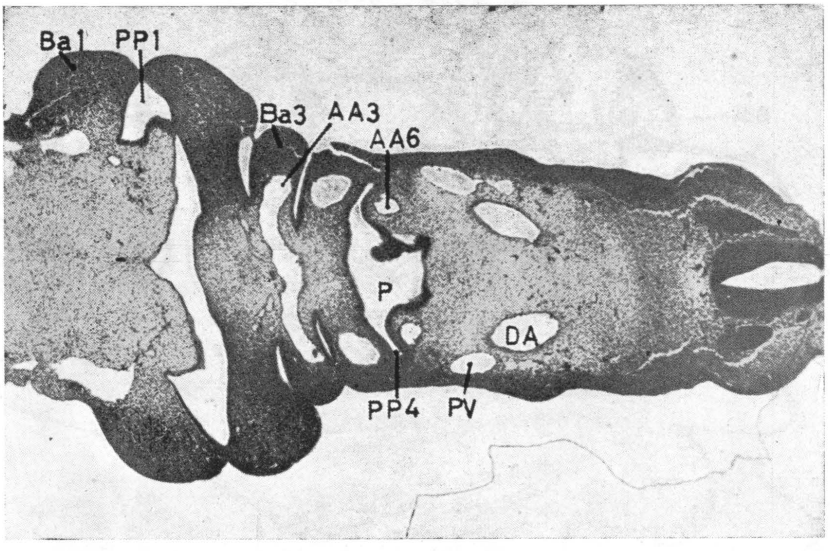

FIG. 9.—A photograph of a section through the pharyngeal region of a sheep embryo showing the sequence of the pharyngeal pouches, branchial and aortic arches (23 days). × 30.

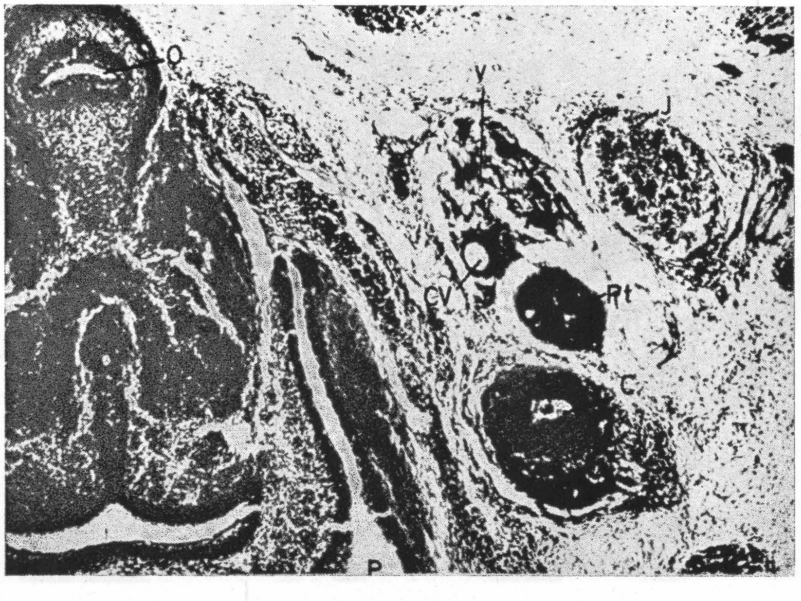

FIG. 10.—A photograph showing relations of cervical vesicle, parathyroid, carotid, ganglion nodosum and jugular vein as described on page 200 of text (31 days). × 75·6.

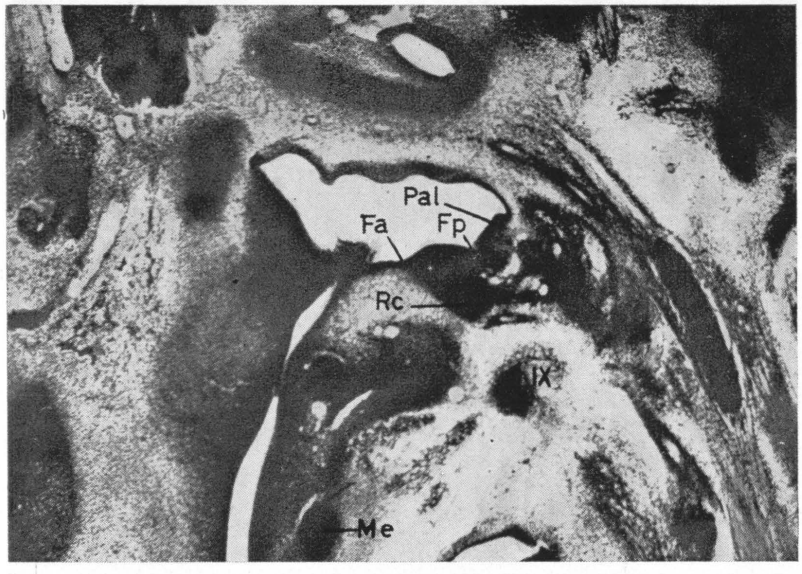

FIG. 11.—A photograph of a sagittal section of an embryo showing the anterior and the posterior fossa tonsillaris separated by an intertonsillar fold (28 days). × 30.

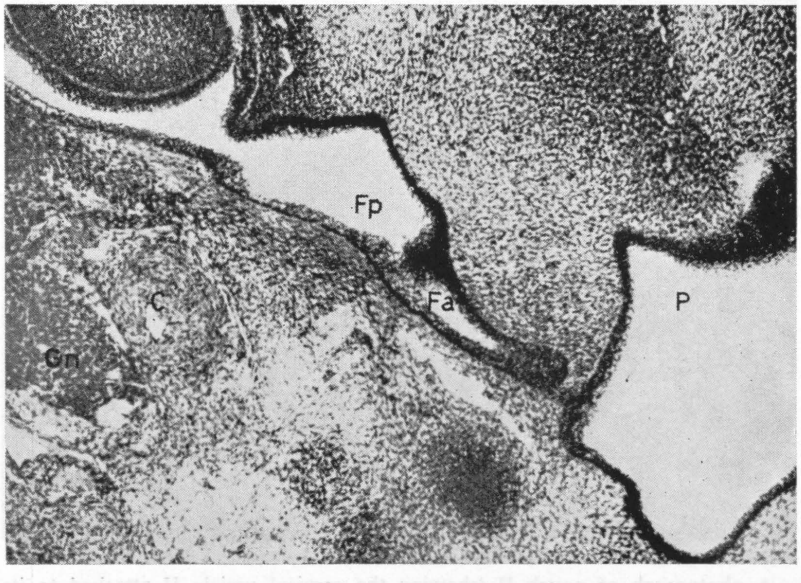

FIG. 12.—A photograph of a transverse section of an embryo showing the anterior and the posterior fossa tonsillaris (28 days). × 75·6.

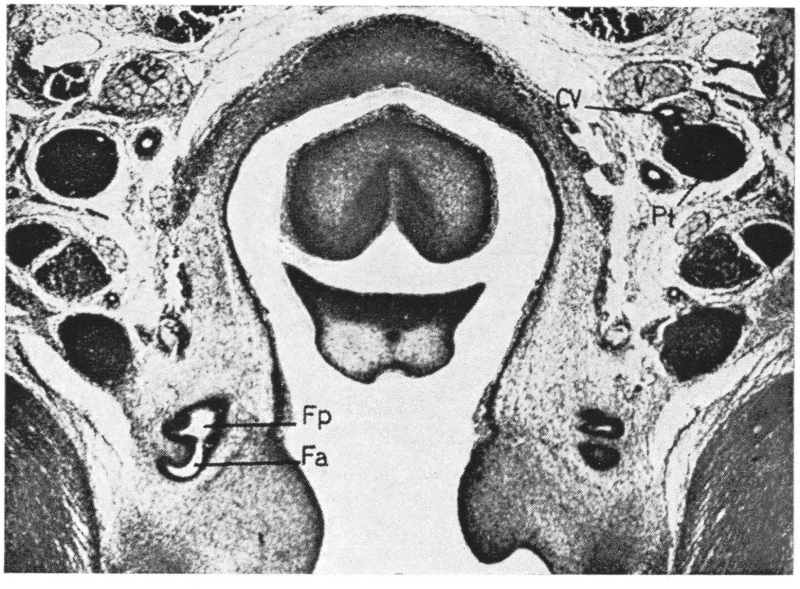

FIG. 13.—A photograph of a transverse section through the arytenoid swellings showing the union of the anterior and posterior fossae to form the sinus of the palatine tonsil. Deeper down (on right side) the fossae are separate. Parathyroid III and cervical vesicle IV attached to the cranial end of the thymus cord are also shown (34 days). ×30.

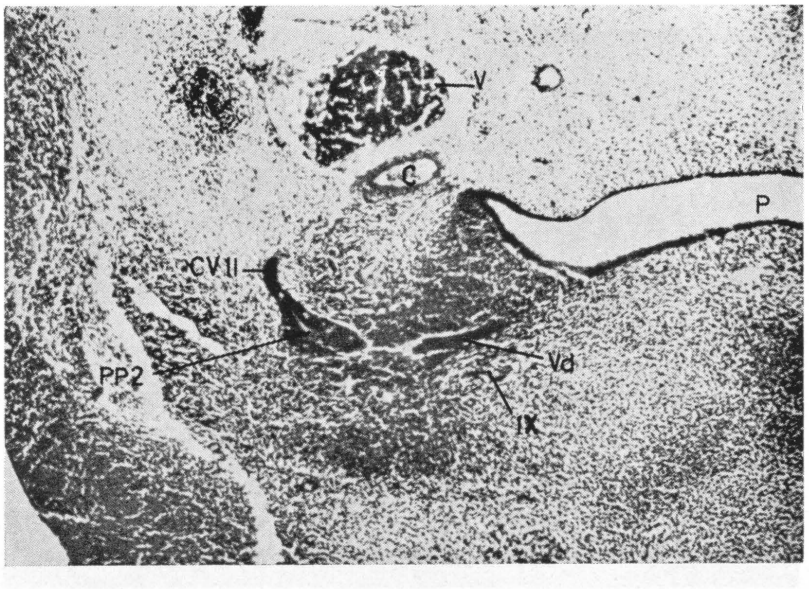

FIG. 14.—A photograph of pouch II (showing the cervical vesicle II attached to its caudo-lateral pocket). The ventral diverticulum is seen apart from the pharynx and pouch II (26 days). × 75·6.

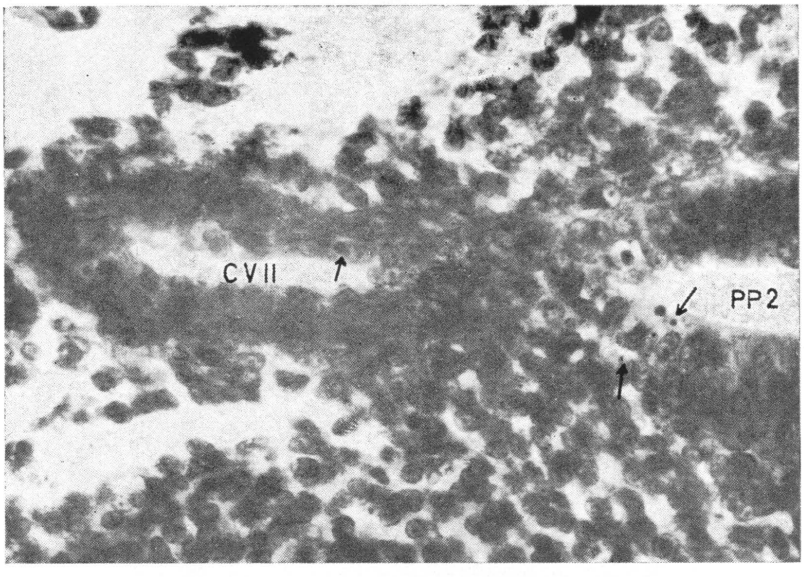

FIG. 15.—A photograph showing contact of the cervical vesicle II and the pharyngeal pouch II (its caudolateral pocket). Cell debris (arrows), present as a result of their regression is only faintly shown due to lack of depth (± 25 days). × 480.

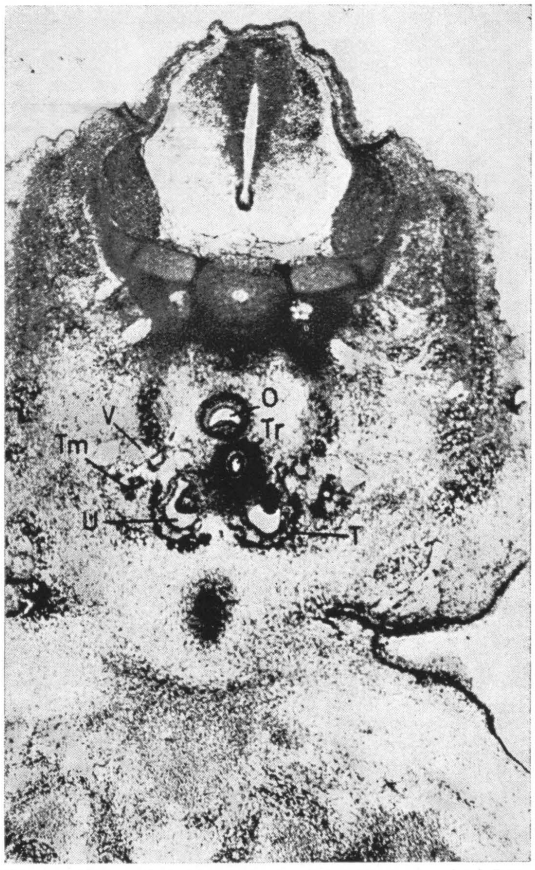

FIG. 16.—A photograph of a transverse section showing the relations of the oesophagus, trachea, thyroid, ultimobranchial body, thymus, jugular vein and carotid (28 days). × 30.

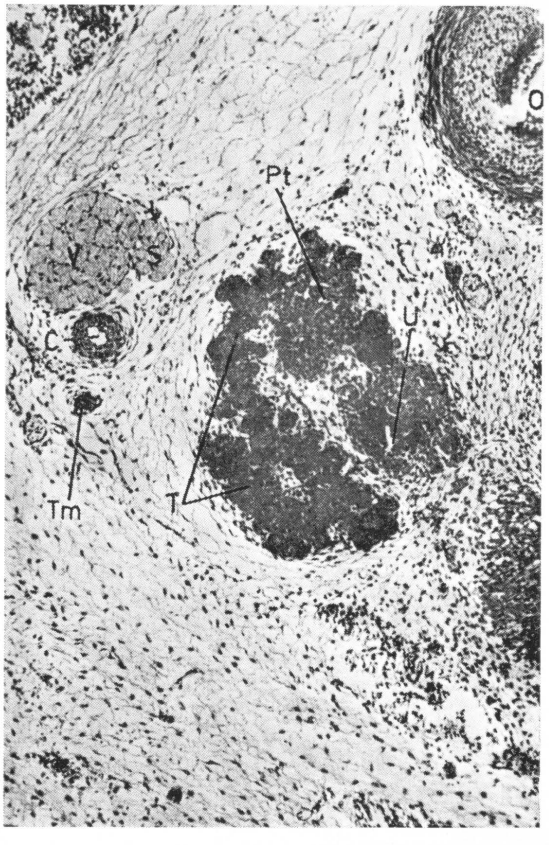

FIG. 17.—A photograph of a transverse section of an embroyo showing the parathyroid IV and the ultimobranchial body surrounded by the thyroid (36 days). × 75·6.

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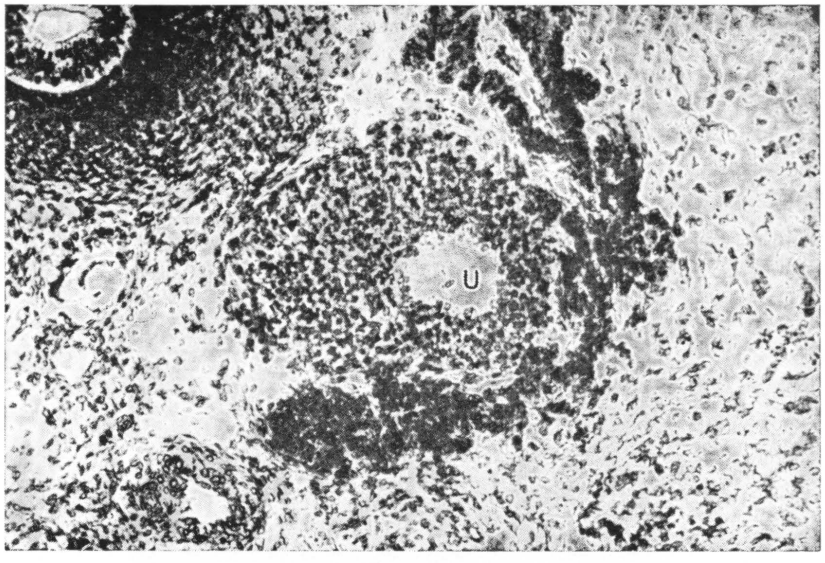

FIG. 18.—A photograph of a transverse section showing the caudal end of the lumen of the ultimobranchial body divided into two by a thin partition (36 days). × 96·8.

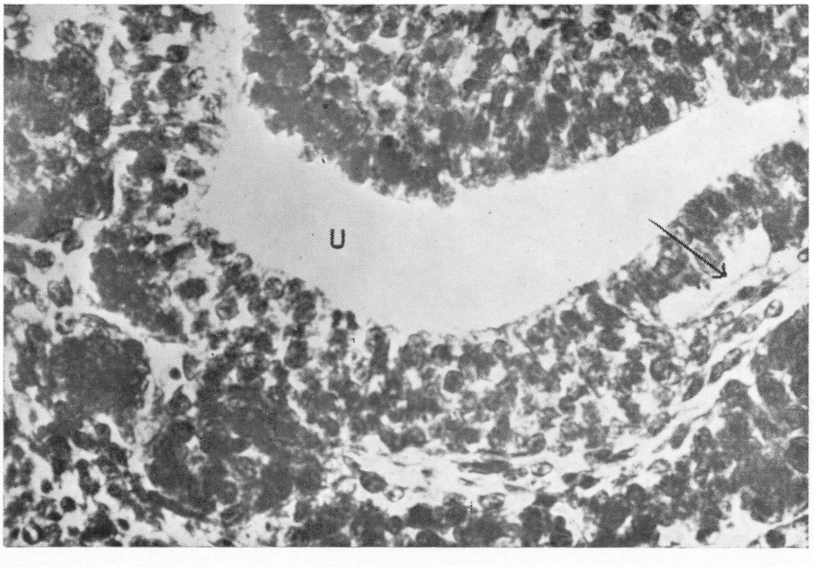

FIG. 19.—A photograph of the ultimobranchial body showing its stratified epithelium containing a group of clear cells (36 days). × 192.

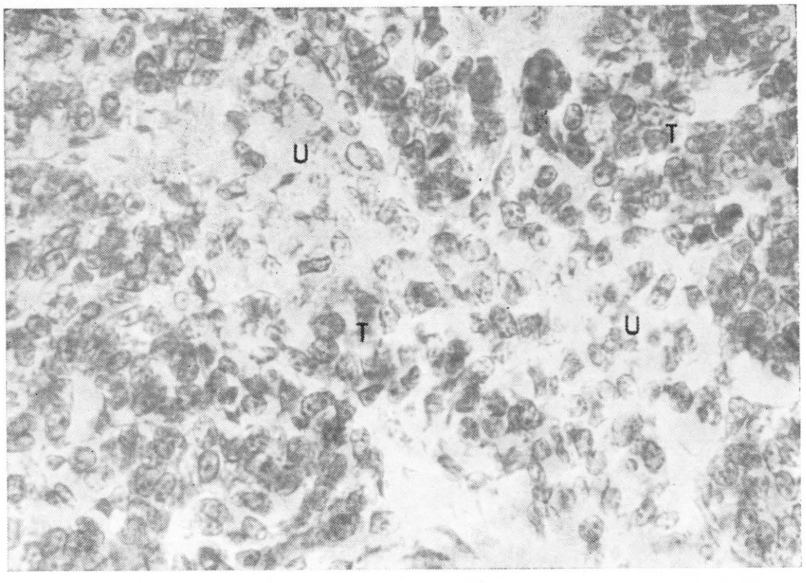

FIG. 20.—A photograph of the cell cords of the ultimobranchial body (clear) intermingling with those of the thyroid (36 days). × 480.

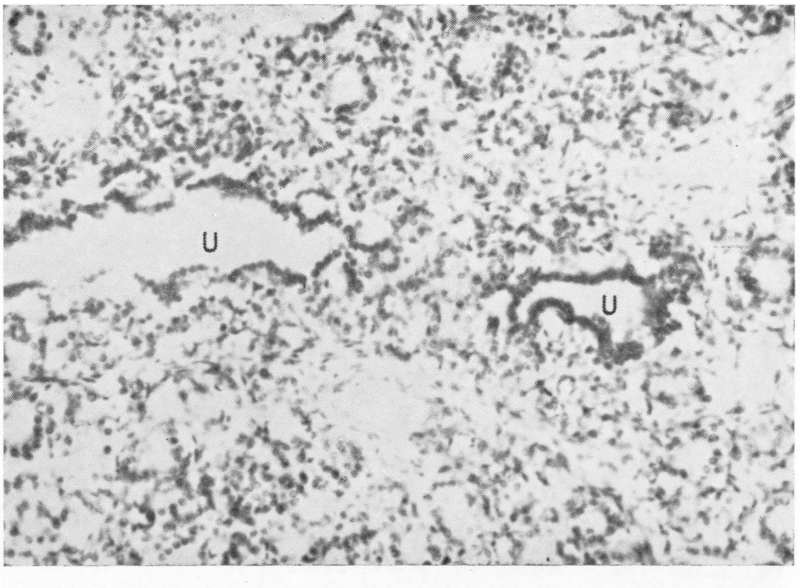

FIG. 21.—A photograph of the thyroid with a "central canal of Prenant" showing follicle formation from it by budding. All follicles are filled with pink-staining colloid although only faintly visible in photo (80 days). × 75·6.

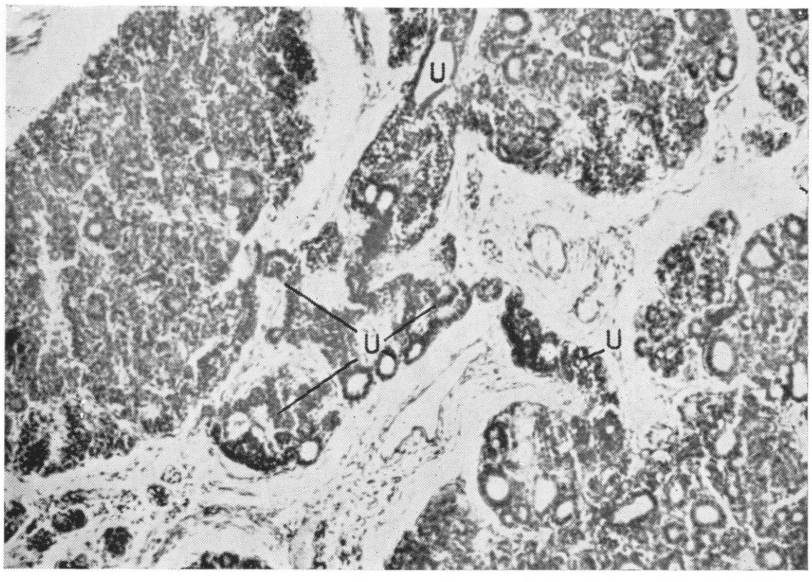

FIG. 22.—A photograph of the thyroid showing cell cords of the ultimobranchial body merging with those of the thyroid—being a typical example of reserve tissue. In contrast to the previous these follicles had not yet formed colloid (80 days, 195 mm). × 96·8.

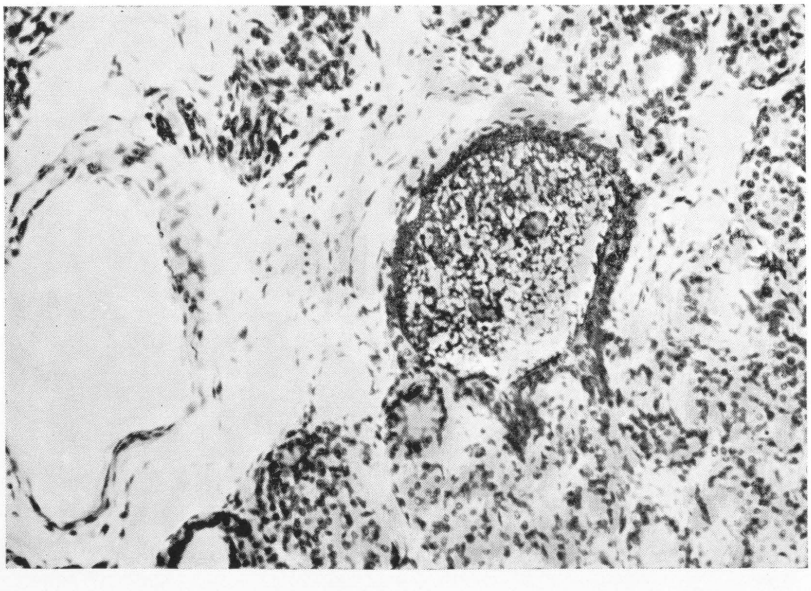

FIG. 23.—A photograph of the thyroid showing a mucoid cyst giving rise to follicles by proliferating cell cords. This cyst was attached to the caudal end of the incorporated parathyroid (92 days, 255 mm). × 192.

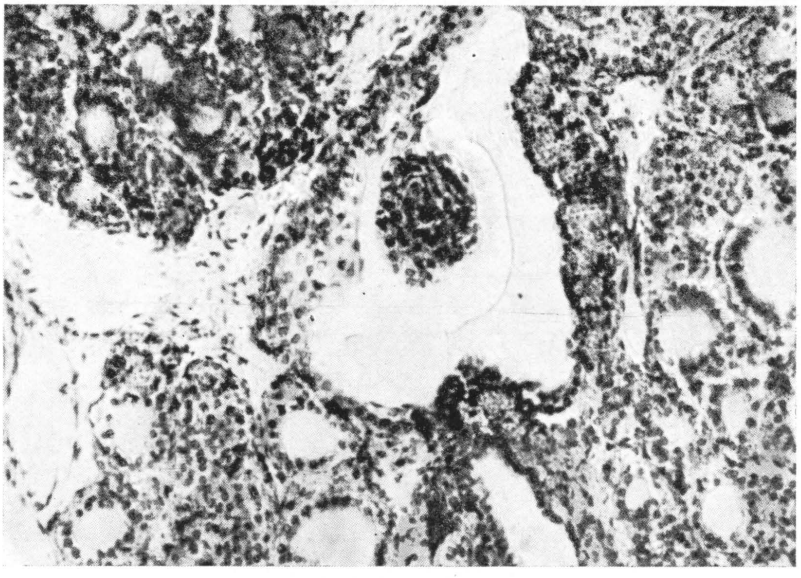

FIG. 24.—A photograph of a transverse section through the ultimobranchial body showing transformation of the cuboidal, colloid-forming epithelium into stratified, squamous, non-colloid-forming epithelium where connective tissue is increased around it (92 days, 255 mm). × 192.

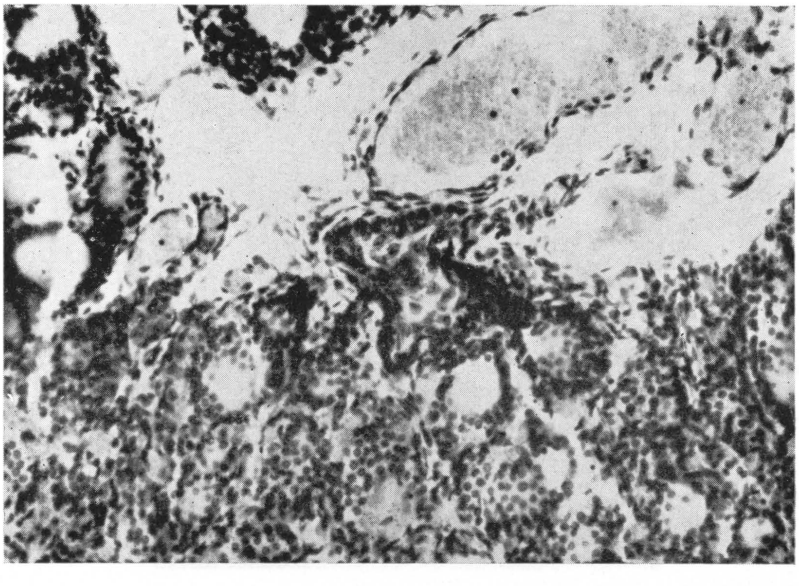

FIG. 25.—A photograph of an early dermoid cyst derived from the ultimobranchial body and showing proliferating cords (92 days, 255 mm). × 192.

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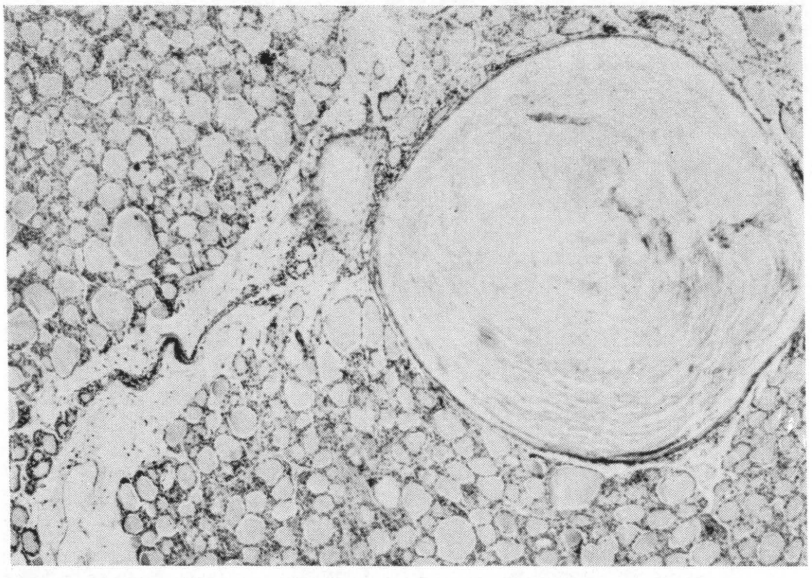

Fig. 26.—A photograph of a transverse section through an adult dermoid cyst (also with proliferating cell cords transforming into follicles) which has become expanded by accumulation of desquamated epithelial cells. × 75·6.

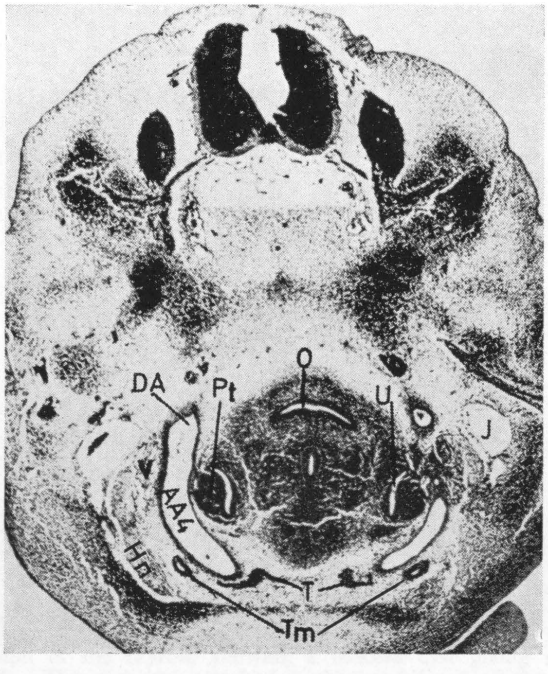

FIG. 27.—A photograph of a transverse section at the height of the 4th aortic arch (future aorta) showing the various relationships (26 days, 15 mm). × 30.

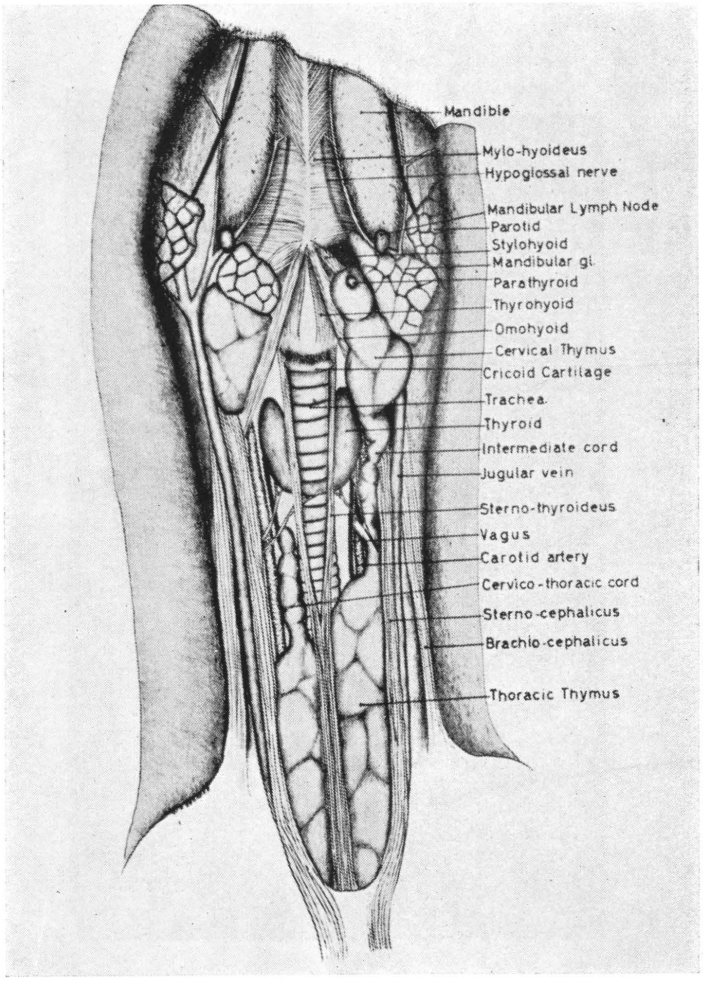

FIG. 28.— Dissection of the cervical region of a sheep foetus showing the normal relations on the left side (of the photograph). On the right side the mandibular gland has been tilted sideways and the head of the cervical thymus drawn ventrally to show the position of the parathyroid III. On the left side the intermediate cord is situated below the sterno cephalicus and the internal carotid (130 days).

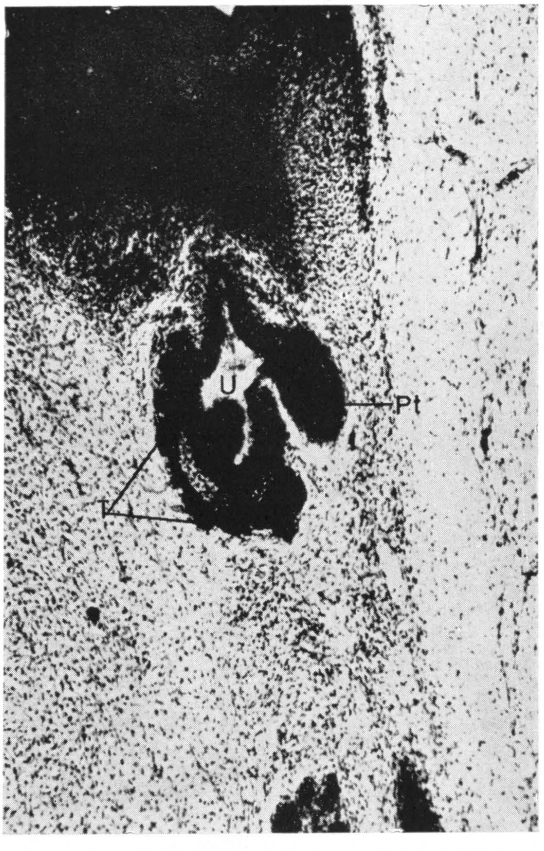

FIG. 29.—A photograph of a sagittal section through the complex IV, incorporated craniomedially into the lateral thyroid lobe (29 days, 21 mm). × 75·6.

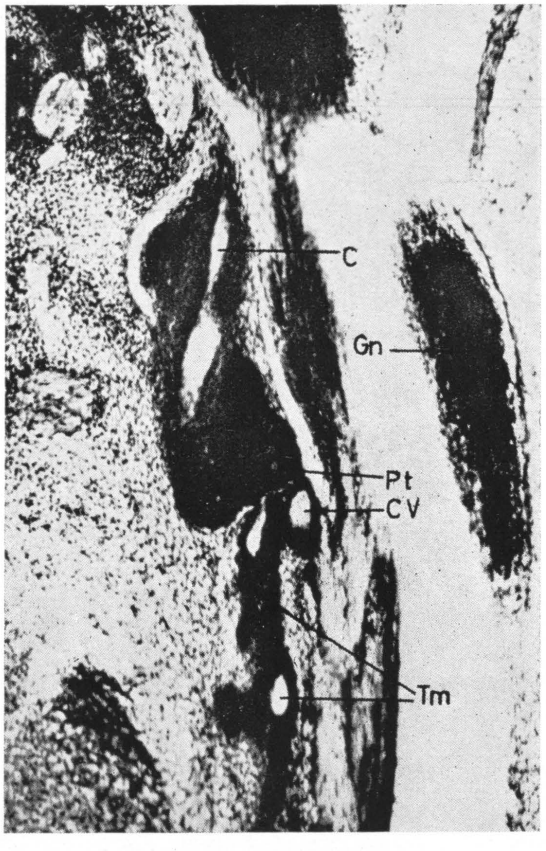

FIG. 30.—A photograph of a sagittal section showing the cervical vesicle fused to the cranial tip of thymus III caudal to the parathyroid (29 days, 21 mm). × 75·6.

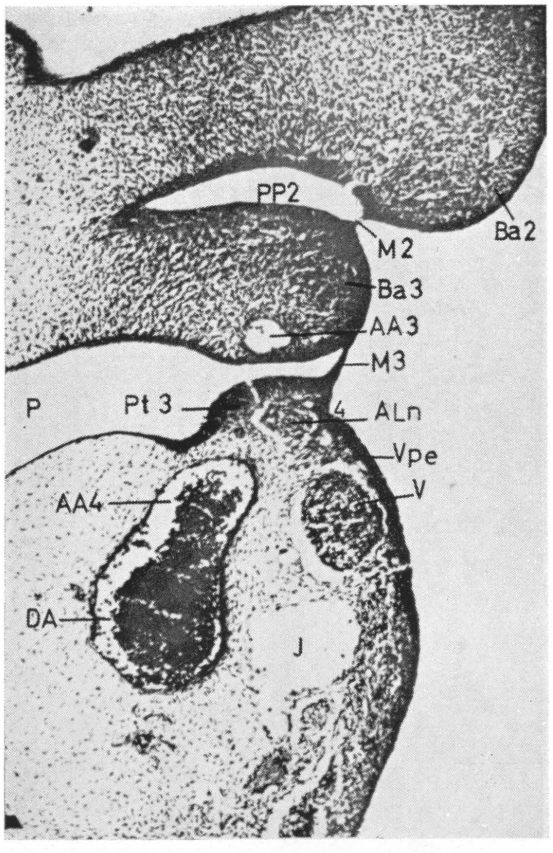

FIG. 31.—A photograph showing the region caudal to the branchio-pharyngeal membrane III from which the cervical vesicle IV is formed. This region is closely associated with the vagus and its pharyngeal (laryngeal) branch (23 days, 23·5 hours). × 75·6.

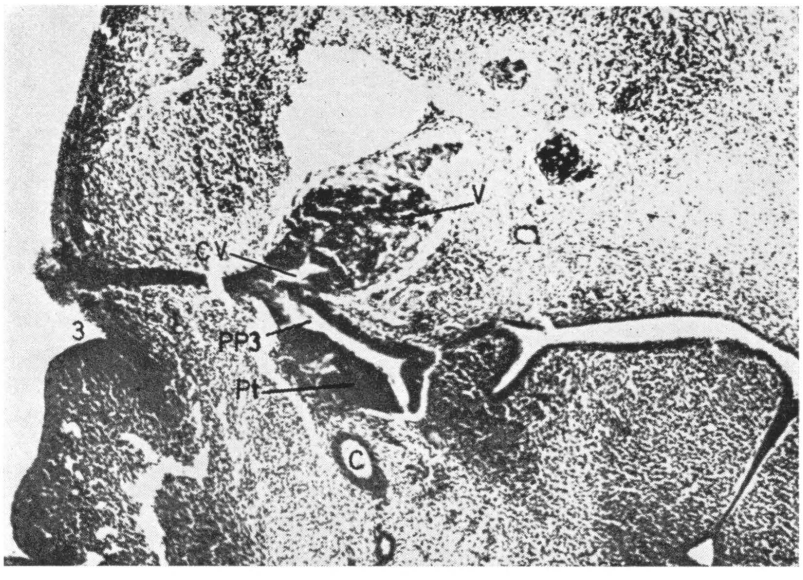

FIG. 32.—A photograph showing the cervical vesicle IV attached (*a*) to a region posterior to cleft 3 by a cell cord (broken) (*b*) to the ganglion nodosum and (*c*) to the lateral part of the pharyngeal pouch III which is being severed from the pharynx (26 days). × 75·6.

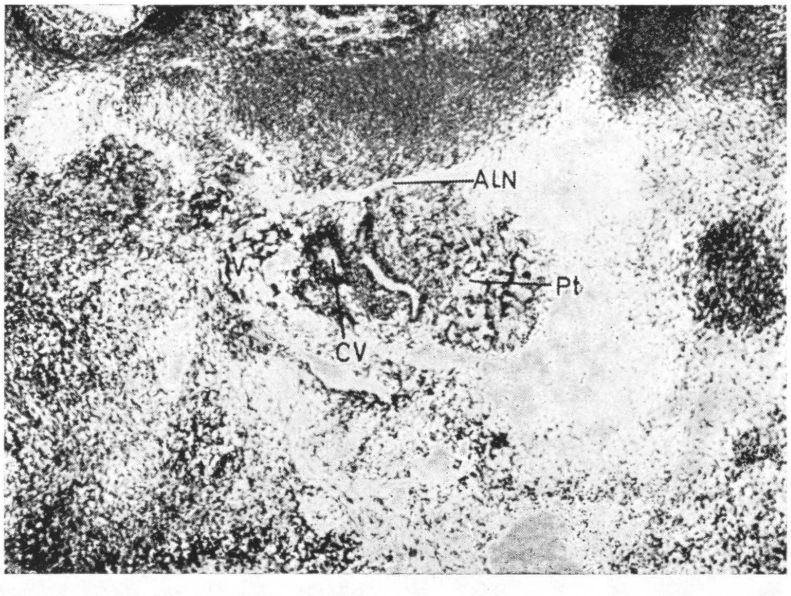

FIG. 33.—A photograph showing the cervical vesicle associated with the ganglion nodosum dorsally and ventrally with the lateral part of pouch III which has been severed from the pharynx by the anterior laryngeal nerve (28 days. × 75·6.

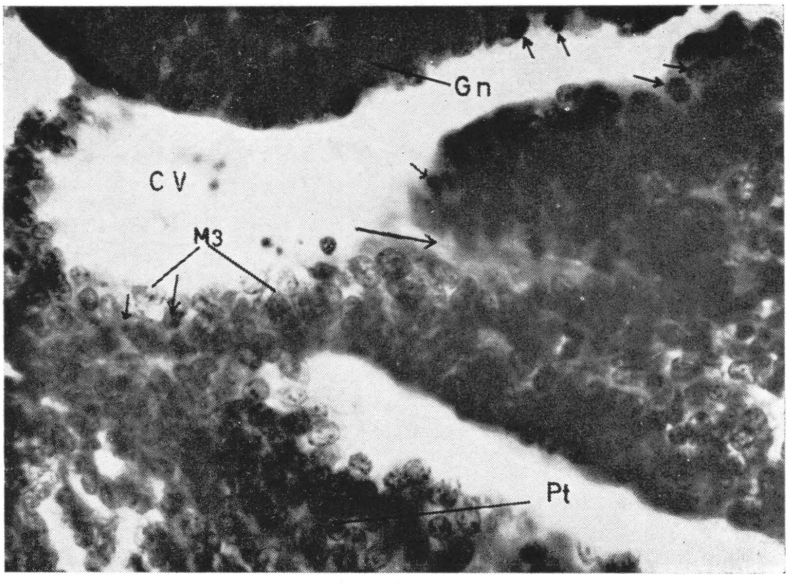

FIG. 34.—A photograph of the cervical vesicle showing a slight diverticulum representing an addition of the 3rd branchial cleft (large arrow) both being separated from the pharyngeal pouch III by the closing plate. The latter shows cell debris (small arrows), a sign of degeneration whereas the cervical vesicle itself shows active mitoses (small arrows) ($24\frac{1}{2}$ days). × 480.

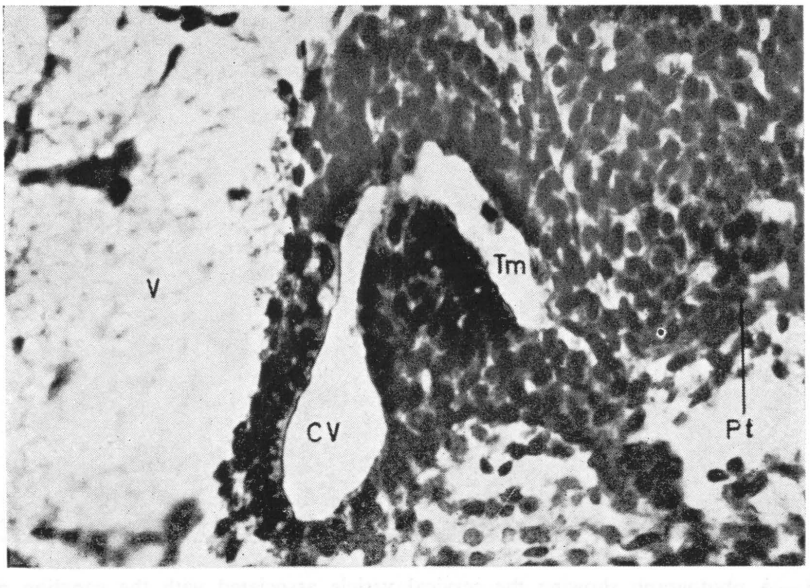

FIG. 35.—A photograph showing the lumen of the cervical vesicle joined to that of the thymus cord (29 days). A remnant of the pharyngeal membrane 3 is just seen. × 480.

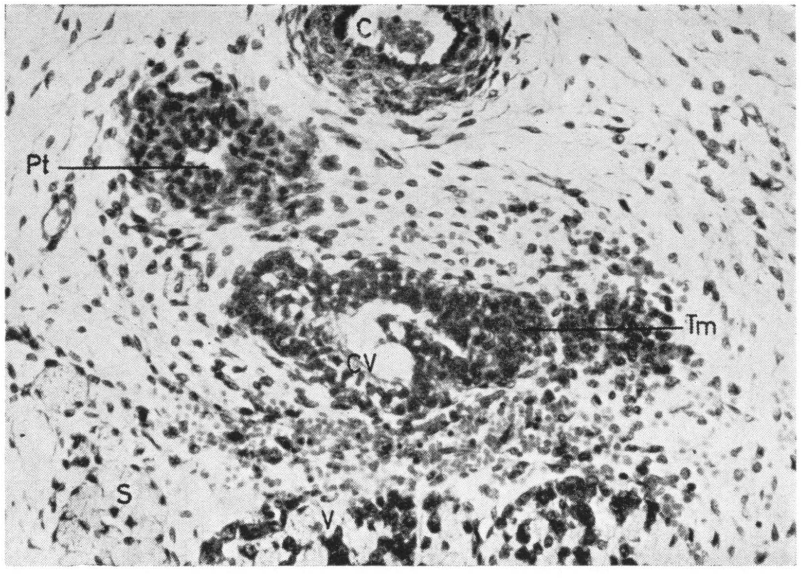

FIG. 36.—A photograph of a later stage than the previous showing the cervical vesicle and thymus cord sharing a common lumen. The cervical vesicle shows clear cells typical for early stages of keratinising epithelium. No thymocytes have formed here although most surrounding mesenchymal cells show round instead of oval nuclei. More caudally lymphocytes were formed in mesenchyme. This is a transverse section at the height of the caudal end of the parathyroid (36 days). × 192.

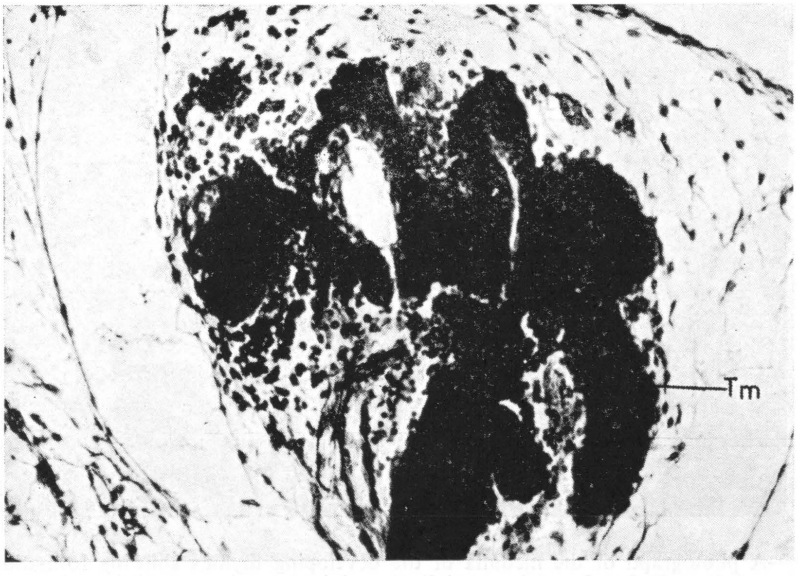

FIG. 37.—A photograph of a later stage than previous showing thymus cords stained intensely due to invading thymocytes which are also numerous around cords, often appearing in small groups (40 days). × 192.

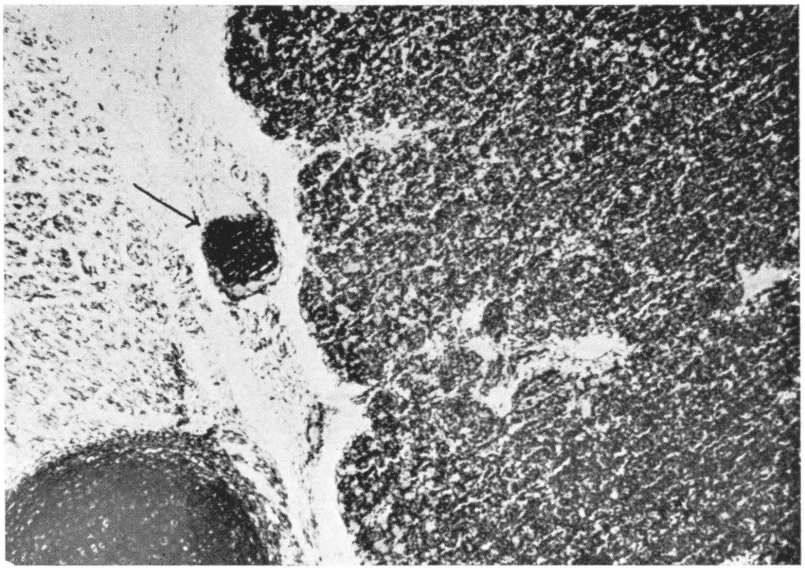

FIG. 38.—A photograph of the only structure encountered which could possibly develop into a thymus IV. It was connected by its caudal end to the medial aspect of the thyroid indicating its possible origin as remnant of the ultimobranchial body (63 days, 125 mm). × 75·6.

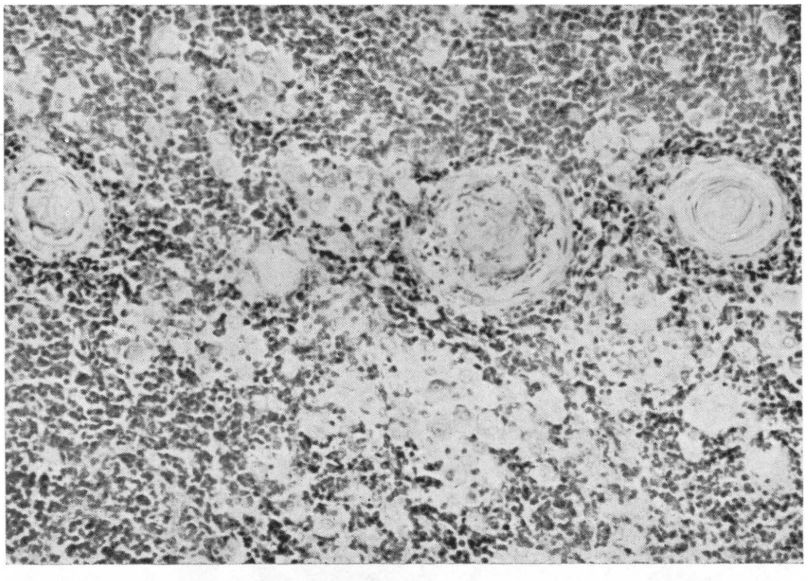

FIG. 39.—A photograph of the medulla of the developing thymus showing formation of the corpuscles of Hassall, i.e. from the partially keratinised portion of the thymic cords. The proximally situated cells of these cords become separated by invading lymphocytes. These separated cells can become whorled to form new corpuscles of Hassall in later stages (106 days, 310 mm). × 192.