THE HISTOGENESIS OF THE

BRANCHIAL CYST

BY

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INTRODUCTION

The branchial cleft cyst is most often found in adults, twenty to forty years of age, situated just below the angle of the mandible, anterior to the sternocleidomastoid muscle. (7,36) Varying types of lining epithelium, all of which are usually intimately related to lymphoid tissue with germinal centers, contribute to its identification as a unique cyst. However, the question of its histogenesis has been a matter of controversy for some time. The most popular hypotheses implicate the branchial apparatus, (1,48) the thymic duct, (62) and parotid salivary gland duct inclusions in lymph nodes. (7)

The purpose of this investigation is to examine data which would tend to support or reject these hypotheses, with particular reference to the salivary gland duct inclusion theory. This theory postulates the genesis of most branchial cysts from parotid salivary gland duct tissue trapped in cervical lymph nodes during development. The observation of salivary gland duct inclusions in parotid lymph nodes, the histologic finding of lymphoid tissue in the sub-epithelial connective tissue of surgically excised branchial cysts, and certain clinical observations on patients with branchial cysts have led some to the hypothesis that these cysts develop from salivary gland duct inclusions in cervical lymph nodes.

The most important phase of this investigation was to examine a series of surgically excised branchial cysts with particular reference to the various types of epithelium lining them in an attempt to explain their presence in terms of the more popular hypotheses on the histogenesis of these cysts. The second aspect of this investigation was to determine the frequency of occurrence of ductal epithelium in cervical lymph nodes at various levels of the neck. The following basic concepts, on which there is general agreement, should be considered as important elements of this study:

1) Ductal epithelium is enclaved in some lymph nodes in the face and neck, particularly in the parotid gland area.

2) This ductal epithelium may undergo proliferation and subsequent cystic change.

3) Ductal epithelium has been observed to undergo squamous metaplasia in some inflammatory conditions.

4) Respiratory epithelium in various organs has been observed to undergo squamous metaplasia in some inflammatory conditions.

5) Ductal epithelium has not been observed to undergo metaplasia to respiratory epithelium in inflammatory conditions.

6) Respiratory epithelium has not been observed to undergo metaplasia to ductal epithelium in inflammatory conditions.

It follows then, that if branchial cysts originate from ductal epithelium, they should be lined by recognizable ductal epithelium, stratified squamous epithelium, or a transitional type of epithelium. Certainly, they should not be lined by obvious respiratory epithelium.

Consider next the following generally accepted concepts:

1) The branchial apparatus normally gives rise to many structures containing various types of epithelium including stratified squamous, respiratory, transitional, ductal, and acinar.

2) Epithelial remnants of the branchial apparatus are concentrated in the areas in which branchial cysts occur most frequently.

It follows then, that if branchial cysts originate from the branchial apparatus, any combination of stratified squamous, respiratory, transitional, or ductal epithelium may be found.

Although the majority of branchial cysts are reported to be lined by stratified squamous epithelium, mixed types of lining epithelium have been seen in some cysts.

These combinations, which consist of varying proportions of stratified squamous, transitional, and respiratory epithelium, suggest that the stratified squamous component arises in part, at least, from metaplasia of pre-existing respiratory epithelium or by direct maturation of the original multipotent epithelium.

The studies reported in the literature have not described ductal epithelium as lining the walls of branchial cysts. Therefore, in order to establish or reject the hypothesis that branchial cysts develop from salivary gland duct inclusions, the following hypothesis which was proposed by Bernier and Bhaskar, will be tested:

> Branchial cysts develop from parotid salivary gland duct inclusions in lymph nodes.

The hypothesis will be tested by the assumption that the number of cysts found to have ductal epithelium (parotid in origin) lining them will be greater than the number of cysts lined by respiratory epithelium (non-parotid in origin). If this hypothesis is rejected, it would seem logical to state that the cysts probably do not develop from salivary gland duct inclusions.

REVIEW OF THE LITERATURE

Embryology

Branchial apparatus. A large part of the neck and pharynx develops from the embryonic branchial or visceral arches. (1,48) The branchial arches are bar-like ridges, separated by grooves or clefts, which appear on the ventrolateral surfaces of the head region of the embryo during the fourth week of intra-uterine life. Four arches are seen superficially; the fifth and sixth arches are submerged. By the beginning of the seventh week of intra-uterine development most external traces of the branchial arches have disappeared. Each branchial arch contains a cartilaginous core, blood vessels (aortic arch, connecting the dorsal and ventral aortae), a nerve, and mesodermal tissue that will give rise to muscle tissue.

An internal space is present which is surrounded by entoderm and represents the primitive pharynx; evaginations from the primitive pharynx separate the branchial arches from each other. The external surfaces of the branchial arches are covered by ectoderm, and invaginations are present, forming clefts or grooves which separate the arches externally.

The ectoderm of the branchial cleft and the entoderm of the corresponding pharyngeal pouch finally come into contact, with little or no mesoderm lying between them, forming the closing membrane. (18, p.227) In fish and some amphibians this membrane ruptures and gill slits result. In man the arches fuse and finally present a smooth inner and outer surface.

The first branchial arch is separated into maxillary and mandibular portions. It gives rise to the lateral portion of the upper lip, upper jaw, lower lip and jaw, body of the tongue, Meckel's cartilage and adjacent soft tissues. (18, p.75; 47, p.429) The malleus and incus develop from the proximal end of Meckel's cartilage. The anterior part of the mandible develops from the distal portion and the intermediate part never ossifies but forms part of the anterior ligament of the malleus and portions of the spheno-mandibular ligament. (50)

The second branchial arch gives rise to the body of the hyoid bone, archus glossopalatinus, hyoid ligament, hyoid muscles, anterior portion of the base of the tongue, the lesser wing of the hyoid bone, and adjacent soft parts. Also, the proximal portions of the second skeletal cartilage bar, termed Reichert's cartilage, takes part in the formation of the facial canal. (5)

The third branchial arch gives rise to the greater cornu of the hyoid bone, posterior portion of the base of the tongue, archus pharyngeal palatinus, and surrounding soft tissue. The fourth, fifth, and sixth branchial arches give rise to the cuneiform cartilage, thyroid cartilage, cricoid cartilage, and soft parts in mid and lower neck region. (18, p.244-253)

The structures that arise from the pharyngeal pouches are lined by entoderm. The first pharyngeal pouch gives rise to a portion of the tympanic cavity and the eustachian tube. The eustachian tube of a near-term fetus is found to have a large amount of sub-epithelial lymphoid tissue. The roof of the tube is found to be covered by non-ciliated columnar appearing epithelium. The rest of the tube is lined by pseudostratified ciliated columnar epithelium, (24) And many goblet cells are found in the epithelium near the pharyngeal orifice. (65) The second pouch gives rise to the palatine tonsil and the tonsillar and supratonsillar fossas. The third pouch gives rise to the inferior parathyroids and the thymus gland. From the fourth pouch the superior parathyroid glands develop, and some feel that a small portion also aids in the development of the thymus gland. (2, p. 233) The fifth pharyngeal pouch gives rise to the ultimo-branchial body, which is thought to be involved in the formation of the thyroid gland. (61) (Fig. I)

The branchial grooves or clefts are for the most part completely replaced by mesoderm. However, the first cleft forms the external auditory canal, tragus, and helix of the external ear. (18, p. 298.)

A brief description of the cervical sinus or precervical sinus of His should be considered in order to better understand the fate of the branchial clefts, particularly clefts two, three, and four.

At the 5 mm. embryonic stage of development the second branchial arch and the thoracic body extend more laterally than the third and fourth branchial arches. This forms a depression called the cervical sinus, which can be separated into rostral and caudal portions. The floor of the cervical sinus at the 5 mm. stage is formed by the lateral aspects of the third and fourth branchial arches. (Fig. 2) The shallow second, third, and fourth branchial clefts open into the cervical sinus. The second and third branchial arches begin to expand

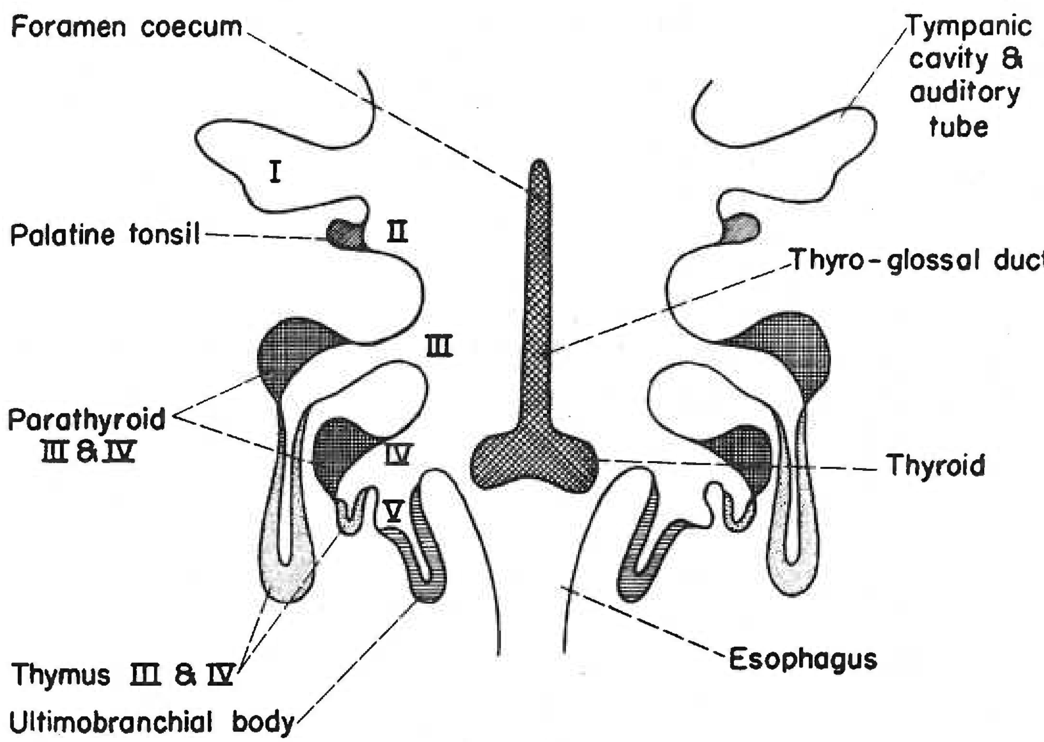


Fig. 1. Diagrammatic outline of the pharynx, at about the stage of 10 mm., to indicate its derivatives. I-V, Pharyngeal pouches.

laterally and caudally, thus approaching the rostral surface of the bulging thoracic body wall. (Fig. 2B) This converts the cervical sinus into two narrow slits. The first is located between the second and third branchial arches and is termed the rostral cervical sinus. The second lies between the third branchial arch and the thoracic body and is termed the caudal cervical sinus. The caudal cervical sinus branches into the third and fourth branchial clefts at its inner aspect. (Fig. 2B) At the 9 mm. stage little separation is present between the third and fourth clefts, and the fourth has rotated to appear as an appendage of the rostral wall of the third cleft. (Fig. 2D) The caudal cervical sinus is gradually obliterated and the slit remaining represents the rostral cervical sinus. (19) (Figs. 2E & 2F)

Development and post-natal cycles of the palatine tonsil. The tonsillar pillars are derived from the second and third branchial arches by a dorsal extension of the mesenchyme from these arches into the soft palate. The pillars are at first composed of mesenchyme covered by entodermal epithelium. Later, the mesenchyme differentiates into the muscular tissues of the pillars. The primitive tonsillar fossa is located in the second pharyngeal pouch. (18, p.262; 47, p.537)

The earliest epithelial covering of the pharynx consists of a single layer of cuboidal cells (9 mm. stage). In the 25 mm. embryo the epithelium is composed of two layers: the basal layer, which is definitely columnar, and a more superficial layer. At the 40 mm. stage of development the tonsillar fossa can barely be seen with the naked eye. The pharyngeal epithelium at this time consists of four rows of cells. By the time the embryo reaches the 75 mm. stage of development, the pharyngeal epithelium has five rows of cells, and the surface layer

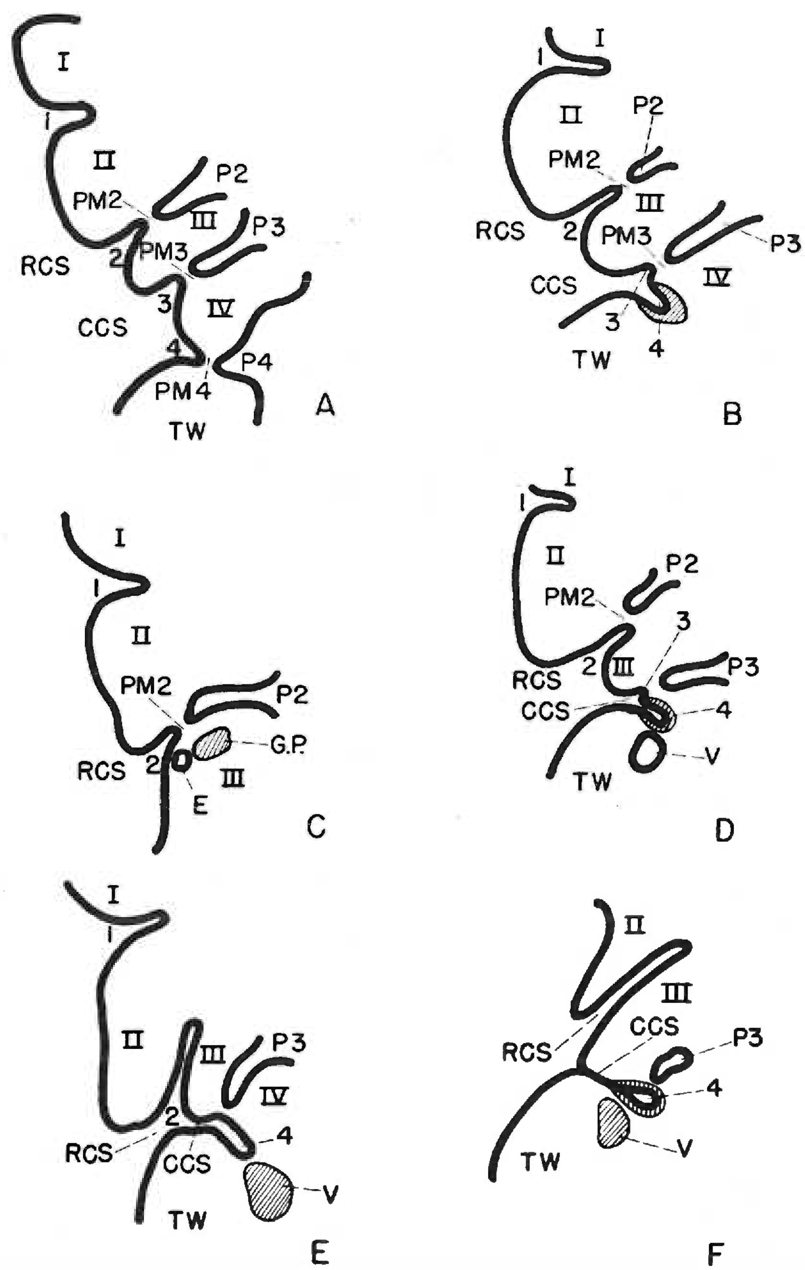


Fig. 2. Redrawn from photomicrographs of Garrett.[3]

A. Human embryo (6 mm.). Section through the pharyngeal region showing the primitive relationships between branchial arches (Roman numerals), clefts (Arabic numerals), and the tips of the corresponding pharyngeal pouches (P 2, 3, 4). x 52.

B. Human embryo (8 mm.). A similar section showing the thickening of the walls of the fourth cleft (4), the regression of the third cleft (3), and the narrowing of the cervical sinus (CCS, RCS). x 52.

C. Human embryo (8 mm.). A section through the same embryo 0.90 mm. nearer the brain, showing an early stage (E) in the formation of vesicle 2. x 52.

D. Human embryo (9 mm.). Section similar to those of Figs. 1 and 2 showing narrowing and deepening of the fourth cleft (4), disappearance of the third cleft (3), and further constriction of the cervical sinus (CCS, RCS). x 52.

E. Human embryo (10.5 mm.). A similar section showing modification of the fourth cleft (4) and constriction of the cervical sinus (CCS, RCS). x 52.

F. Human embryo (12 mm.). Shows the closure of the fourth cleft, transforming it into vesicle 4 (4) by obliteration of the caudal portion of the cervical sinus (CCS). x 52.

possesses cilia. Columns of epithelium begin to extend from the surface of the primitive tonsil downward into the submucosal mesenchyme. These epithelial columns soon show swelling and degeneration of the cells in their centers. By this process of degeneration and liquefaction of the central portions of the columns of epithelium, they become hollow or cystic. In this way the tonsillar crypts are formed. (44; 46, p.120) The formation of crypts continues until about the time of birth. In the 98 mm. embryo the surface epithelium of the pharynx is well ciliated throughout. Even the tongue and esophagus have ciliated epithelium at this time. (46, p.121)

Weber's mucous glands can be seen developing in fetuses as small as 75 mm. These glands are formed by a downward protrusion into the sub-epithelial mesenchyme by the basal layer of the low columnar surface epithelium. These then develop as slender tubular structures. (46, p.122)

The tonsillar fossa is clearly visible by the sixteenth week of fetal development (115 mm). The first sign of the developing tonsillar parenchyma is seen at the 115 mm. stage. Small islands of newly-differentiated lymphocytes accumulate around the crypts, deriving from the mesenchyme in the area. (46, p. 122-125) Even when the fetus is at full term, the number of lymphocytes in the tonsil is small compared to the amount found two months after birth. (44)

The muscle tissue surrounding and entering into the formation of the tonsil is derived from the mesenchyme in this region by a process of differentiation. In the first stage of muscle formation the mesenchymal cell elongates and becomes finely granular, possessing only a single nucleus located in its center. This future muscle cell increases in length and begins to acquire fibrils and more nuclei. The myofibrils push the nuclei toward the periphery as they increase in number. The muscle cell of the newborn has fewer striations than the muscle cell of the adult. (46, p.127)

The connective tissue in the 98 mm. fetus is not yet of a mature type. Young fibroblasts have developed thin fibrils, but are still a definite embryonic type. As lymphocytes form from the mesenchyme, they push the fibrils of the immature connective tissue together. The long axis of the fibroblasts thus arrange themselves parallel to what will eventually be the surface of the tonsillar capsule. This primitive capsule of young fibroblasts will mature to form the tonsillar capsule. It has been said that the tonsil has no true capsule, and that the structure usually termed the capsule is in reality the adjacent submucosa. (46, p. 127-129)

The migration of lymphocytes through the epithelium can be seen just before birth. However, the two main features of the adult tonsil are not present until after birth. Plasma cells are first seen at about the third week of postnatal life. The secondary nodule is first apparent sometime during the third to sixth postnatal month. (44, 46, p.128)

Just after birth the tonsil is still quite small; however, it soon begins to increase in size due to the infiltration of plasma cells and lymphocytes. Secondary nodules with germinal centers soon become apparent. It is difficult to say what constitutes a normal tonsil for any certain age group because of the various infectious factors which have acted on any given tonsil. Parkinson (46, p.133) feels that from the fourth decade on there is a tendency for the tonsil to decrease in size.

Development of the pharyngeal tonsil. In the early fetal stages the primordium of the pharyngeal tonsil appears as a series of longitudinal ridges formed by undifferentiated mesenchyme raising up the overlying entodermal covering in the nasopharynx.

The cilia noted on the surface of the epithelium of the pharynx disappears by the time the fetus reaches 115 mm. in length. However in the nasopharynx the epithelium remains ciliated and is pseudostratified. (46, p.137) Thus, the epithelial covering of the pharyngeal tonsil is of a pseudostratified ciliated columnar type.

Observations of Arey (2, p. 239) record the presence of lymphocytes in the pharyngeal tonsil of 100 mm. fetuses. Secondary nodules develop in the adenoids a few months after birth. The surface of the pharyngeal tonsil is increased by a system of longitudinal folds rather than by means of the crypt system that prevails in the palatine tonsil.

Development of the thyroid. Tourneup and Verdun (58) concluded that the thyroid gland arose from one medial and two lateral bodies which developed from the ventral surface of the primitive pharyngeal cavity. They believed that the medial component made its first appearance at about the 3 mm. stage of embryonic development. Weller (61) also states that the thyroid is formed from a medial component and two lateral components. He described the first evidence of the median component as a proliferation of pharyngeal entoderm in the midline of the ventral wall of the primitive pharynx (between the second and third branchial arches). This small mass of cells then extends down into the underlying mesenchyme, forming the first evidence of the thyroglossal duct. The thyroglossal duct, thus, is the analage of the median portion of the thyroid gland. The origin of the thyroglossal duct is from the area of the ventral wall of the pharynx between the portions that will give rise to the tuberculum impar and the copula of the tongue. The thyroglossal duct is Y-shaped by about the 8.5 mm. stage due to proliferation of its cells and also to the growth of the surrounding mesenchyme. By the 13.5 mm. stage an Isthmus and two lateral lobes can be seem at the distal end of the thyroglossal duct. The connection of the thyroglossal duct with the pharynx is lost a short time after its downward migration into the neck region has begun. By the 14-15 mm. stage, the median primordium is U-shaped, and the structure is composed of two layers of somewhat cubodial cells in close approximation.

At about the 5-7 mm. stage the two entodermal projections are noted extending from the lateral portion of the primitive pharynx from the region of the fifth pharyngeal pouches. (45) These structures are thought by Weller (6) to represent the lateral components for the thyroid gland. Some authors (26,37) term these structures the ultimobranchial bodies, and question their relationship to the development of the thyroid gland. In the embryonic material studied by Weller (61) at about the 12 mm. stage, each lateral thyroid primordium was shaped like a long-necked gland and had a para-thyroid primordium close by.

The primordia for the lateral components of the thyroid gland become separated from the pharynx at about the 16-17 mm. stage.

Grosser (26) felt that the term "lateral thyroid anlagen" was a misnomer for the so-called ultimobranchial bodies, and questioned the relationship between them and the development of the thyroid gland. Kingsbury (37) states that the ultimobranchial body is formed as a result of continued growth activity of the branchial endoderm, having no association as an ancestral gland. He states that it can be clearly identified within the thyroid gland, but finally disappears in man without leaving a trace or giving a clue as to its function.

The studies of Weller (61) appear to establish a direct relationship between the so-called lateral thyroid components or ultimobranchial bodies and the histogenesis and development of the thyroid gland. Also, several clinical cases in which autopsy material has been examined would tend to add additional support to this concept.

A thirteen year old white female (autopsy #2855, John Hopkins Hospital), who was a myxedematous idiot during life, revealed several very interesting findings at autopsy. (61) Examination of the neck revealed the absence of a thyroid gland in the normal position. Sagittal sections of tissue at the root of the tongue revealed thyroid tissue measuring 5 mm. in diameter. Also noted in the lateral neck regions were two 2 mm. cystic structures. These structures, one on each side, were both associated with a parathyroid gland. Microscopically these were composed of thyroid tissue with colloid appearing material in the lumens. It was felt that these two cystic structures represented the lateral thyroid primordia and not aberrant masses of thyroid tissue, because of the paired character, location, and associated parathyroid glands.

Other developmental variations of the thyroid gland evidenced during both childhood and adult life seem to substantiate the embryological findings of Weller in the development of this gland. Marshall, (40) in a study of thyroid glands from sixty children whose deaths were apparently not related to the thyroid gland in any way, noted wide variations in the development of the thyroid, many of which seemed to support the idea of the thyroid arising from three primordia, two lateral and one medial.

Fusion of the median and lateral components of the thyroid takes place by growth, establishment of the structures of the neck, and the relative elevation of the head. The median component differentiates before the lateral components. Complete differentiation of the lateral components is completed by about the 40 mm. stage of development. (61)

Development of the parathyroid glands. The parathyroid glands also develop as entodermal evaginations from the primitive pharynx. (18, p.262 47, p.235; 61) The superior parathyroid glands develop from the region of the fourth pharyngeal pouch, and the inferior parathyroid glands develop from the region of the third pharyngeal pouch. The inferior parathyroid glands arise in close association with the thymus gland.

Development of the thymus gland. The thymus gland develops from the third pharyngeal pouches and possibly a small portion of the fourth pouch, according to Arey. (2, p.233) Weller (61) found evidence of the developing thymus primordia after the median thyroid component had begun to develop, but before the lateral thyroid component could be identified. The two components of the thymus meet in the midline at about the 20 mm. stage of embryonic development. As with the thyroid primordia, the connections of the thymic ducts with the pharyngeal mucosa are lost quite early (by the time embryo is 14.5 mm. in length). The descent of the thymus along with the closely associated inferior parathyroid glands to a position inferior to the thyroid gland explains the change in relationship of the inferior and superior parathyroid glands in regard to origin and adult positioning.

Differentiation of the thymus results in a series of scallops and tongue-like elongations of the entoderm, which becomes infiltrated by lymphocytes. Thus, the cortex of the thymus is composed of lymphocytes and the medulla of epithelium. (4)

Development of the salivary glands. All of the salivary glands arise in a similar manner due to the invagination, or ingrowth, of oral epithelium into underlying mesenchyme.

The major salivary glands are generally regarded as being derived from stomodeal ectoderm. The minor salivary glands arise from either entodermal or ectodermal epithelium depending upon their location. There appears to be no histological difference between the minor salivary glands arising from pharyngeal entoderm and stomodeal ectoderm. (47, p.476)

The parotid gland is the first of the salivary glands to make its appearance in the developing embryo. Around the sixth week of embryonic development an epithelial invagination can be seen on the inner surface of either cheek. (17; 47, p.476) The 26 mm. embryo shows the parotid primordium to consist of a narrow duct-like cord of cells with a central lumen. At the 47 mm. stage the parotid primordium elongates and extends backward to a position just anterior to the ear. By the 91 mm. stage the parotid consists of a freely branching system of ducts with no apparent capsule. (17) The 138 mm. embryo reveals a lobular parotid, with primitive acini.

Lymphatic tissue can be seen in the area of the developing parotid. While some investigators question whether these collections of lymphocytes should be considered lymph nodes, (57) others accept them as such. (35, 36)

Occasionally, parotid salivary gland duct inclusions are found in these lymph nodes. (6, 36, 41) The parotid gland during its embryonic development, unlike the submaxillary and sublingual salivary glands, does not have a definite capsule separating it from contiguous tissues. Hence, some of the glandular tissues may become separated from the developing gland and become entrapped in the lymphoid tissue developing in the same region. (17) One investigation found that nineteen newborn infants (autopsied) had one or more lymph nodes in the parotid region which contained salivary gland duct inclusions. (10)

The submaxillary glands are first seen late in the sixth week of embryonic development as paired primordia, consisting of cords of epithelial cells. Each cord, arising near the mid-line under the tongue, represents the main duct of the gland on that side. The duct grows back along the floor of the mouth, near the angle of the mandible and then turns ventrally. It then grows toward the surface, pushing outside the border of the mylohyoid muscle where it begins to branch freely, forming the gland proper. (2, p.222; 47, p.477) The proximal portion of the submaxillary duct is formed by a modification of the floor of the mouth, anterior to the origin of the primordium of the gland. (18, p.243)

Thompson and Bryant (57) observed the lack of lymphoid tissue in the developing submaxillary gland. Also, they observed that the integrity of the submaxillary gland during development was well maintained.

The sublingual glands arise slightly later than the submaxillaries, Their primordia are usually recognizable by the end of the seventh week. The sublingual glands are really a secondary grouping of a row of small glands which arise independently. Their secreting portions merge, more or less, within a common connective-tissue investment, but they retain their original ducts, each gland opening by a row of some ten to twelve ducts which empty onto the floor of the mouth on either side of the roots of the tongue. (18, p.244; 47, p.478) Lymphoid tissue has not been described as being associated with the developing sublingual glands.

Development of the tongue. The first evidence of the development of the tongue is a small swelling noted on the pharyngeal surface of the ventral portion of the second branchial arch. This swelling is first seen between the 5-7 mm. stage of development. By the 8 mm. stage the swelling (tuberculum impar) noted on the second branchial arch has increased in size and has two lateral swellings from the first branchial arch associated with it. By the 16 mm. stage the third arch begins to swell in the mid-ventral pharyngeal region forming the copula of the tongue. The origin of the thyroglossal duct can be traced to a position between the tuberculum impar and copula. The foramen cecum is formed by the growth of the copula and tuberculum impar, and may mark the location of the origin of the thyroglossal duct but can hardly be said to be the remnant of the duct. The sulcus terminalis marks the forward extent of the posterior portion of the tongue (copula, or portion derived from the third branchial arch). (18, p.238-244; 47, p.434-7)

The lateral swellings or processes soon grow over the tuberculum impar and form the anterior portion of the tongue. The anterior portion of the tongue begins to develop papillae during the third month of intra-uterine life. The fungiform and filiform papillae develop a short time before the circumvallate papillae. The papillae of the tongue are formed by circular downgrowths of the entodermal lining covering the dorsal surface of the developing tongue. These invaginations subsequently hollow out. Taste buds make their appearance at about the same time as the papillae, first noted as thickened zones in the entoderm and ectoderm. It has beem said that the taste buds are widely distributed by the sixth month, covering the tongue and surrounding structures, even the soft palate. The greater number of taste buds seem to degenerate about the time of birth. (18, p.241)

The growth of the tongue to its full size is not only a matter of increase in size of the four primitive processes, but also is in a large part due to the development of muscle tissue. The muscle tissue appears to develop from mesenchymal cells located in the epipericardial ridge. These primitive cells are continuous with the occipital myotomes and may have developed from them. These cells are also in close association with the hypoglossal nerve.

By the 6 mm. embryo stage, these future muscle cells are found as two masses in the mandibular region. At first the masses lie deeply under the epithelial surface of the developing tongue, and soon grow up into the enlarging primitive tongue to continue their development into mature muscle tissue. (18, p.241)

Branchial Fistulas And Cysts

The branchial cyst and the branchial fistula are non-serious conditions which may be seen in medical and dental practice. The main clinical concerns are the cosmetic appearance, the inflammatory involvement with discharge resulting in limited discomfort to the patient, and the problem of differential diagnosis of more serious conditions, such as metastatic carcinoma, which may involve the life of the patient.

Branchial fistulas. Branchial fistulas occur as often in males as in females, are usually seen at birth or under the age of five, and tend to occur unilaterally, though bilateral occurrence is not uncommon. They may also be secondary, resulting from the drainage of a cyst or so-called sinus tract. Branchial cysts may be found anywhere along the path of a branchial fistula.

The usual tract passes between the internal and external carotid arteries at the bifurcation, and if the fistula opens internally into the pharynx it will lie close to the fossa of Rosenmueller. If it opens externally, however, its exit is usually in close association with the anterior border of the sternocleidomastoid muscle in the lower third of the neck. (22, 23, 31, 33, 34, 54, 64)

Branchial fistulas are classified into three types by Hoover.(31) The complete fistula has its external opening above the clavicle lateral to the midline, and its internal opening into the pharynx near the lower portion of the tonsillar fossa. The second type is an incomplete fistula with only an external opening, and the third an incomplete fistula with only an internal opening.

The most common symptoms seen in association with branchial fistulas are: continuous or intermittent discharge of mucous, recurrent attacks of inflammation with periodic obstruction and cyst formation, and vagus symptoms produced on palpation. (33, 48, 52, 60) Some patients state that fistula drainage will increase when they have an upper respiratory infection. (52, 60) The only other common draining cervical sinus tract is that occurring secondarily to tuberculous adenitis.

Branchial fistulas or sinuses should be excised when discovered, except in the very young or in the presence of active inflammation. (14, 55)

Branchial cysts. Branchial cysts most commonly occur between the ages of twenty and forty. However, they can be seen anywhere from birth to ninety years of age. (36, 48)

Branchial cysts can be found on either side of the neck or may be bilateral. (36, 52, 53) They are found in close association with the anterior border of the sternocleidomastoid muscle and never appear behind or posterior to the muscle. (39) The cysts may be found along the anterior border of the muscle; however, the majority are found opposite the middle third of the muscle. (7, 36, 39, 52)

The cysts may bulge into the floor of the mouth or the base of the tongue, but seldom into the pharynx. They may lie superficial to the carotid artery, (near its bifurcation, most often) in the bifurcation of the carotid artery, or deep to the carotid artery. (60)

There appears to be some familial tendency to develop branchial anomalies. (23, 60) Bailey (3) reports on five patients in whose families one or more members had similar lesions.

Branchial cysts attract the attention of the patient by filling with secretions, becoming infected, developing sinus tracts, or by having associated fistulas with openings onto the skin or into the pharynx. The cysts may vary in size from a few millimeters to as large as 5-7 centimeters in diameter. They may lie close to the vagus nerve and, on palpation of the cyst, vagus symptoms of coughing, nausea, and variations of the heart rate can occur. (39)

Surgically removed branchial cysts may be small to large, firm to soft, unilocular or multilocular, and the contents may range from a clear fluid containing some cholesterol crystals to a pulpaceous, semi-solid material. (22, 29, 36, 48) Bailey (3) feels that finding cholesterol crystals in a lateral neck swelling is diagnostic for a branchial cyst.

Histological examination reveals the cyst to be lined by epithelium which is usually stratified squamous, although it may vary in type to a simple cuboidal epithelium. The rare columnar epithelium seems to be restricted to the cysts found near the pharynx.

Approximately ninety-five percent of the branchial cysts demonstrate lymphoid tissue beneath the lining epithelium. (7) In some instances a thin layer of connective tissue separates the lymphoid tissue from the epithelium. The lymphoid tissue in these cysts is variable, both in amount and in organization. In some, the lymphocytic elements are diffusely scattered, while in others, structures closely resembling lymph nodes are seen. King (36) feels that the distribution of the cysts corresponds more closely with distribution of the lymph nodes in the lateral neck region than with any other feature.

Conditions which may mimic the branchial cyst include the following: Non-specific lymphadenitis originating from inflammatory conditions in superiorly located structures, thyroglossal duct cysts, dermoid cysts, cystic hygromas, tuberculous adenitis, carotid body tumors, cat-scratch disease, aneurysms, aberrant thyroid tissue, lymphomas, metastatic neoplasms, Warthin's tumor, and other salivary gland tumors involving the submaxillary or parotid glands. (43, 48, 57) (See Table I)

The treatment of bramchial cysts is enucleation and excision of the entire cyst. Branchial cysts do not recur after complete surgical removal, but may recur after incomplete removal.

Histogenesis Of Branchial Cysts

Branchial hypothesis. Numerous studies postulating the relationship of the branchial cysts to the branchial arches and cervical fistulas are found in the literature. Rathke, (Eighteen hundred and twenty-five) who worked with pig embryos, and Von Baer, (Eighteen hundred and twenty-seven) who worked with humam embryos, contributed basic material which enabled Acherson (Eighteen hundred and thirty-two) to postulate the relationship of branchial cysts to the embryonic branchial arches. Heusinger, (28) (Eighteen hundred and fifty-four) Von Langenbeck (59) (Eighteen hundred and fifty-five) and Bland-Sutton (9) (Nineteen hundred and twenty-two) added material which supported the theory that branchial cysts developed from remnants of the branchial arches found in the young embryo.

TABLE 1 BRANCHIAL CYSTS - DIFFERENTIAL DIAGNOSIS

Lesion	Test For Differential Diagnosis
Non-specific lymphadenitis	A. Rule out infection in oral and pharyngeal regions B. Often more than one node is affected
Thyroglossal duct cyst	A. Midline in location B. Biopsy-- microscopic appearance diagnostic
Dermoid cyst	A. Often midline in location B. Usually attached to overlying skin C. Biopsy-- microscopic appearance diagnostic
Cystic hygroma	A. Young age group B. Often very extensive lesion C. Seldom restricted to site of branchial cyst D. Biopsy-- microscopic appearance diagnostic
Tuberculous lymph node	A. Stains-- acid-fast may be positive in tissue sections B. Culture C. Tuberculin skin test D. Biopsy-- microscopic appearance highly suggestive
Cat-Scratch disease	A. History of cat contact B. Intradermal test C. Biopsy-- microscopic appearance suggestive
Carotid body tumors	A. Deeply seated B. Older age group C. Cannot be displaced in vertical direction D. Biopsy-- microscopic appearance diagnostic
Aneurysm	A. Expansile palpitation B. Aspiration for blood C. Subjective symptoms
Salivary gland tumors	A. Location B. Subjective symptoms C. Biopsy-- microscopic appearance diagnostic
Metastatic tumors	A. Palpation-- often very firm B. Often fixed C. Biopsy-- histology often diagnostic
Lymphomas	A. Multiple lesions (often) B. Subjective symptoms C. Biopsy-- microscopic appearance diagnostic
Benign tumors	A. Biopsy-- microscopic appearance diagnostic
Aberrant thyroid	A. Biopsy-- microscopic appearance diagnostic

The branchial hypothesis is based on the location of the cysts, presence of sinuses and fistulas, and their supposed relationship to the branchial arches and clefts of the embryo. (1, 48)

Gross (25) postulates that branchial cysts and fistulas arise from branchial cleft remnants. He suggests that the great majority of congenital fistulas of the neck are derived from the second branchial cleft.

Ward, Hendricks, and Chambers (60) state that anomalies of the branchial arch apparatus should be grouped under the heading of branchial abnormalities, and therefore do not accept any particular region of the branchial apparatus as giving rise to the branchial cysts and fistulas. Other authors (19, 29) believe the precervical sinus of His to be the portion of the branchial apparatus most responsible for the cyst and fistula formation.

According to Wenglowski (62) and Frazer, (18) if the cysts develop from branchial arch remnants, they would be restricted to an area above the hyoid bone. These authors find it difficult to explain by the branchial arch hypothesis the cysts which are found below the hyoid bone.

The formation of sinuses on an inflammatory basis after birth is quite understandable. King, (36) however, states that congenital sinus and fistula development has not been explored adequately and should not be used to support the supposed branchial arch origin of the branchial cyst. Findings which he believes are not explained by the branchial hypothesis are the constant presence of lymphoid tissue, the variable presence of thymic tissue, the wide distribution of the cysts, and the relationship of some of the cysts with salivary glands.

In summary, the available data does not establish the branchial hypothesis for the origin of "branchial" cysts and fistulas without leaving some areas open to more study and analysis.

Thymic duct hypothesis. Wenglowski (62) in his studies on the origin of the branchial cyst and fistula, reconstructed models from more than sixty human embryos and concluded that branchial cysts had their origin in the course of the thymic duct. He does not explain the absence of unequivocal thymic tissue in most branchial cysts. Although some cysts are found along the course of the thymic duct, he does not explain the wide distribution of the cysts, especially in the upper neck region.

King (36) reports that the thymic duct does not remain circumscribed in form, its cells becoming inextricably mingled with tissues of the neck during embryogenesis. He does not accept the thymic duct hypothesis because the structure as such is not found in the fetus or adult. Also, other regions of the branchial apparatus may give rise to portions of the thymus gland, according to King.

McNealy's (43) view is that the branchial apparatus cannot leave remnants in the neck below the level of the hyoid bone. In his opinion, the branchial cyst develops from persistent thymic tissue left during its migration to the upper chest region.

Burnet (11) described structures resembling Hassal's corpuscles in the walls of a series of branchial cysts. He hypothesized that lymphoid elements can differentiate into epithelial cells and that this may be the source of the epithelium found lining the lumen of the branchial cyst.

Salivary gland inclusion hypothesis. Bernier and Bhaskar, (7) in a report of 468 lateral neck cysts which had been diagnosed as branchial cysts, found that microscopically the cysts had an epithelial lining and that their walls contained lymphoid tissue of varying density and organization. The cysts were lined by stratified squamous epithelium, pseudostratified ciliated columnar epithelium, or by a combination of the two. The cyst wall in approximately ninety-six percent of the cases contained varying amounts of lymphoid tissue. Their investigation revealed that the lymphoid tissue associated with these cysts demonstrated subcapsular and medullary sinusoids. Clinically, some of the cysts were painful and fluctuated in size when respiratory or dental infections were present. They suggested that this showed a connection between the cysts and the lymphatic system of the head and neck region, and that the pain and increased size of the cyst was due to products of inflammation that drained from a distant site. They also noted that lymph nodes in or near the parotid gland may contain epithelial inclusions.

In another study, (6) Bernier and Bhaskar presented a classification of lymphoepithelial lesions of the salivary glands and postulated that most of them arose from salivary gland duct inclusions within lymph nodes of the parotid and cervical regions. They observed that clinically and microscopically the branchial cleft cyst closely resembled a lesion which they termed the benign lymphoepithelial cyst of the parotid region. They interpreted this cyst as developing from parotid salivary gland inclusions in lymph nodes and suggested that this is also the origin of most branchial cysts.

Bernier and Bhaskar (7) stated that branchial cysts do not arise from the branchial apparatus for the following reasons: 1) lesions identical to branchial cysts occur within the parotid gland, and were interpreted as "cystic" lymph nodes; 2) the appearance of fluctuation associated with local inflammation is difficult to explain if one assumes a branchiogenic origin; 3) the presence of lymphoid tissue around these cysts cannot be explained on the gill arch basis; 4) branchial cysts, unlike branchial fistulas, are seldom if ever seen at birth; 5) branchial cysts are connected neither to the pharynx nor to the skin surface.

The investigators (7) estimated that about 96% of the cysts in their study developed from parotid epithelial inclusions in cervical lymph nodes. About three percent of the "branchial cysts" in their series did not have lymphoid tissue in the cyst wall. It was believed that these lesions probably arose from misplaced epithelium left from organ migration in the cervical region.

MATERIALS AND METHODS

Embryos, Fetuses And Radical Neck Dissections

The first phase of the study is based on the histologic examination of four embryos, thirty-seven fetuses, and forty-nine radical neck dissections. These tissues were examined in an attempt to gain insight into the histogenesis of the branchial cyst. Support to the parotid origin of these cysts would be a high frequency of salivary gland inclusions in lymph nodes located in the same region of the neck in which branchial cysts are most commonly found, the middle neck region. (See Fig.5) Also, the morphology of the lining epithelium of the various body cavities of the head and neck region arising from the branchial apparatus could give some indication of the origin of the epithelium lining the branchial cyst.

Embryos. The embryos were serially sectioned and stained with Hematoxylin-Eosin. Particular attention was paid to the development of the parotid and sub-maxillary salivary glands. The morphology of the lining epithelium of the oral cavity, pharynx, esophagus, eustachian tube, respiratory areas, and the various sinuses of the head was noted.

Fetuses. The stillborn and premature fetuses were prepared in the following manner: One-half of the mandible (with adjacent soft tissue including the parotid gland) and the soft tissue of the neck, was dissected free and fixed in ten percent formalin. The side of

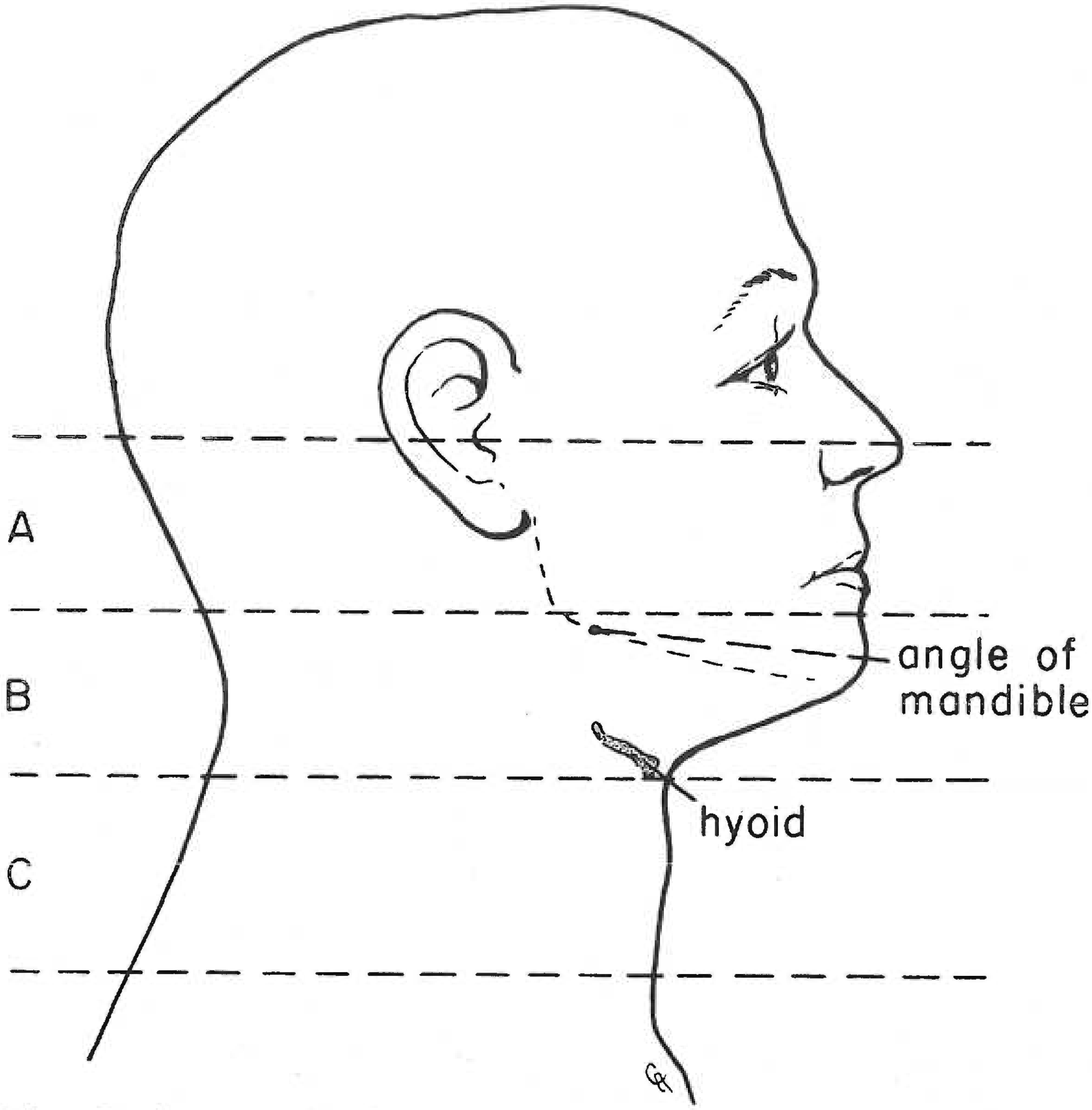


Fig. 5 Anatomical zones of the neck designating the clinical locations of branchial cysts. A-upper neck. B-middle neck C-Lower neck

dissection was alternated from specimen to specimen. The resected specimen was then sectioned and decalcified in a sodium formate and formic acid solution, and Hematoxylin-Eosin, Masson's and Mayer's mucicarmine stained sections prepared. Masson's stain was used in an attempt to identify epithelial rests, and mucicarmine to identify epithelium containing mucicarmine positive material (probably mucin). Because of the bulk of material each tissue block was not serially sectioned, but one slide prepared from each serial block which averaged 6 mm. in thickness (7-15 blocks per case). The areas under study were the various lining epithelia, the anatomy of the parotid, sublingual and submaxillary salivary glands, the lymph nodes throughout the neck, and the presence or absence of epithelial rests in the neck.

Radical neck dissections. The radical neck dissections had been performed during the treatment of patients with various types of carcinoma of the head and neck region. The tissue blocks from these cases were obtained from hospitals in the Portland area and Hematoxylin-Eosin, Masson's and mucicarmine stained slides were prepared. The tissues of interest were the resected lymph nodes and salivary glands. Those nodes found to contain epithelial tissue other than carcinoma were classified as to the region of the neck from which they were removed. Several cases in which the parotid gland was removed for benign conditions were also collected.

Branchial Cysts

The material involved in this second phase of the study consisted of the histories, surgical descriptions and representative Hematoxylin-Eosin, and Mayer's mucicarmine stained slides from 149 branchial cysts. These were part of the original collection of 209 cases listed as branchial cysts in hospital files. For various reasons, however, sixty of these did not meet the criteria for this study. (See Table 2)

One to four unstained slides from each of the 149 cases were also available. The Hematoxylin-Eosin sections were reviewed for the percentage and frequency of occurrence of the different types of lining epithelium. Other items recorded included the presence or absence of inflammation and the relative amounts and organization of lymphoid and glandular tissues in the walls of the cysts.

The clinical histories and operative reports yielded information on most of the patients' age, sex, race, the location of the lesion, the clinical symptoms and the type of treatment rendered.

Classification of epithelial types. The epithelium was classified into the following types: stratified squamous,(Fig. 26B) transitional, (Fig. 40B) respiratory,(Fig. 31B) "probable respiratory," (Fig. 42C) ductal, (Fig. 34B) "probable ductal," (Fig. 39B) and indeterminate (Fig. 36). (See Table 3) The transitional type had the same morphology as that lining the ureter or the epithelium found at the transition of the respiratory epithelium and stratified squamous epithelium in the nasopharynx. The respiratory epithelium was ciliated pseudo-stratified columnar. The "probable-respiratory" epithelium had the same morphology as the respiratory type, but no definite cilia could be demonstrated.

TABLE 2

SUMMARY OF 209 CASES IDENTIFIED IN HOSPITAL FILES AS BRANCHIAL CYSTS

Branchial Cysts	Slides with complete clinical history	138
	Slides with incomplete clinical history	11
	No slides, complete clinical history	4
	No epithelial lining on slides, clinical history	32
	Sub Total	185
Branchial Fistulas and Sinuses	Fistulas with history	1
	Sinuses with history	12
	Sub Total	13
Other Lesions	Thyroid carcinoma	2
	Gastric lined adenomas	5
	Warthim's tumor	1
	Lymphangioma	1
	Transitional cell carcinoma	1
	Bronchogenic cyst	1
	Sub Total	11
	TOTAL	209
Study Includes:	A. Branchial cysts with complete history and slides with epithelial lining	138
	B. Branchial cysts with incomplete history and slides with epithelial lining	11
	Study Group Total	149

TABLE 3 CLASSIFICATION OF THE LINING EPITHELIUM OF BRANCHIAL CYSTS

EPITHELIUM	ABBREV.	MORPHOLOGY	MUCICARMINE STAIN
Stratified Squamous	S	Mature: 1. Varied in thickness 2. With or without keratin formation 3. Squamous cells may be at surface	Negative
		Modified: 1. Lymphocytes and macrophages often in epithelium 2. Epithelium distorted-compressed 3. Squamoid cells at surface - no keratin	Negative
Transitional	T	1. Varied in thickness 2. Occasional goblet-like cells 3. Scalloped surface 4. No squamoid cells at surface	Parts of the lining often positive
Respiratory	R	1. Varied in thickness 2. Often contains goblet cells 3. Pseudostratified columnar 4. Cilia demonstrable	Parts of the lining always positive
Probable Respiratory	PR	1. Varied in thickness 2. Often contains goblet cells 3. Pseudostratified columnar 4. Cilia not demonstrable	Parts of the lining always positive
Ductal	D	Often double row of nuclei, simple cuboidal, or pseudostratified columnar epithelium	Most often negative (lumenal contents often stained positive)
Probable Ductal	PD	1. Varied in thickness 2. Most often pseudostratified 3. Double row of nuclei suggested in some areas	Most often negative
Indeterminate	I	1. Single to multiple cell thickness 2. Appeared undifferentiated or non-specific 3. No classical morphologic pattern	Negative

The ductal epithelium consisted of a double row of nuclei with a columnar type of cytoplasm at the luminal surface. Some pseudo-stratified columnar epithelium was considered to be ductal rather than "probable respiratory" in order to give all possible support to the "ductal inclusion theory" for the origin of branchial cysts. In addition, the "probable ductal" type included those which more closely resembled the ductal epithelium than any other of the classified types. Indeterminate refers to a type of epithelium which could not be designated as stratified squamous, ductal, respiratory, or a more primitive type; it could have been any of these.

Measurement of epithelial types. A Leitz Prado microprojector projected the image of each of the Hematoxylin-Eosin slides onto an eight-by-twelve inch sheet of white bond paper at a distance of three and one-half feet. The lining epithelium was traced on this paper, after which it was classified morphologically by microscopic examination. The respective types of lining epithelium were represented on the tracings by using a different colored pencil for each morphologic type. After all of the tracings had been classified, a Minerva map measurer was used to assess the length of the various epithelial types lining each cyst.

A "reduction" ratio was used to convert the epithelial measurements made from the tracings to represent the actual length of the lining epithelia. The ratio used was the length of the projected image of a cyst to its length as measured directly from the Hematoxylin-Eosin slide and was found to be 8.7. Each of the epithelial measurements from the tracings was divided by this figure. For example, if the projected length of the lining epithelium of a cyst were 17.4 cm.

the actual length of the lining epithelium would be 17.4 cm./8.7 or 2 cm. By simple addition it was possible to tabulate the total length for each of the types of lining epithelium for all of the cysts. From this data the percentage of the various epithelial types was derived for each cyst, individually and collectively.

Basis for mucicarmine interpretations. Sections of both normal and pathologic mucosae which had been removed surgically for conditions other than branchial cysts and occurring in the oral, pharyngeal, nasopharyngeal, and laryngeal regions were stained with Mayer's mucicarmine stain and examined microscopically for the presence of mucicarmine-positive material along with positive control sections. The types of epithelium studied included stratified squamous, transitional and respiratory. It was observed during this survey that stratified squamous epithelium was consistently mucicarmine-negative, while portions of the respiratory epithelium were always positive and portions of the transitional type were often positive. (See Table 4) This finding was helpful in differentiating stratified squamous from transitional epithelial linings of branchial cysts when morphological criteria were insufficient. If the mucicarmine stain were positive, the epithelium was classified as transitional, whereas those with negative findings and questionable morphology for transitional epithelium were classified as stratified squamous epithelium.

The data collected were subjected to appropriate statistical analysis in an attempt to establish or reject the thesis that salivary gland inclusions in cervical lymph nodes give rise to the majority of branchial cysts. These data were based on the morphology of the epithelial lining of resected branchial cysts.

TABLE 4 SURVEY OF MUCICARMINE STAINING REACTIONS IN ADULT ORAL, PHARYNGEAL, NASOPHARYNGEAL AND LARYNGEAL TISSUES

TISSUE	PATHOLOGIC DIAG.	TYPE OF EPITHEL.*	MUCICARMINE STAIN
Nasal	Polyp	R	Positive
Nasal	Polyp	R,T	Positive (Both)
Nasal	Polyp	R,T	Positive (Both)
Nasal	Polyp	R	Positive
Vocal Cord	Chronic Inflammation	S	Negative
Nasal	Polyp	R,T	Positive (Both)
Nasal	Chronic Inflammation	None	Negative
Eustachian	Chronic Inflammation	P.C.	Positive
Tonsil	Chronic Inflammation	R,T	Positive (Both)
Tonsil	Chronic Inflammation	T,S	Positive (Tran.)
Tonsil	Chronic Inflammation	R,T,S	Positive (R,T)
Tonsil	Chronic Inflammation	R,T,S	Positive (R,T)
Larynx	Hyperplastic mucosa	S	Negative
Larynx	Hyperplastic mucosa	S	Negative
Larynx	Hyperplastic mucosa	S	Negative
Maxillary Sin.	Chronic Sinusitis	R	Positive
Vocal Cord	Chronic Inflammation	S,R	Positive (R)
Nasal Septum	Hyperplastic Epithelium	S	Negative
Larynx	Chronic Inflammation	R	Positive
Nasopharynx	Chronic Inflammation	T,R	Positive (Both)
Cheek Mucosa	Hyperplastic Mucosa	S	Negative
Eustachian		I	Positive
Nasopharynx	Hyperplastic Mucosa	S	Negative
Alveol.Ridge	Hyperplastic Mucosa	S	Negative
Buccal Mucosa	Hyperplastic Mucosa	S	Negative
Cheek	Papilloma	S	Negative
Cheek	Fibroma	S	Negative
Cheek	Leukoplakia	S	Negative
Cheek	Leukoplakia	S	Negative
Cheek	Leukoplakia	S	Negative
Cheek	Fibroma	S	Negative
Palate	Papillomatous Hyperplasia	S	Negative
Ant. Pillar	Leukoplakia	S	Negative
Palate	Leukoplakia	S	Negative
Tongue	Sialolithiasis	None	-----
Tongue	Fibroma	S	Negative
Buccal Mucosa	Epulis Fissuratum	S	Negative
Cheek	Focal Keratosis	S	Negative
Palate	Papillomatous Hyperplasia	S	Negative
Palate	Papillomatous Hyperplasia	S	Negative
Cheek	Focal Keratosis	S	Negative
Tongue	Hyperplastic Mucosa	S	Negative
Alveol.Ridge	Hyperplastic Mucosa	S	Negative

* R - Respiratory; T - Transitional; S - Squamous; I - Indeterminate
P.C. - Pseudostratified Columnar.

Thyroglossal Duct Cysts

The third phase of this study consisted of collecting histories, surgical descriptions and representative Hematoxylin-Eosin and Mayer's mucicarmine stained slides from 24 cases of thyroglossal duct cysts. Most investigators agree that the thyroglossal duct cyst arises from epithelial remnants of the thyroglossal duct. Also, it is generally agreed that the thyroglossal duct is of branchial origin. Therefore, the author believed it would be interesting to examine a series of thyroglossal duct cysts to see if they have any histologic similarities to the branchial cyst.

FINDINGS

Embryos, Fetuses, And Radical Neck Dissections

Embryos. A 140 mm. embryo revealed lymphoid tissue in the region of the developing parotid glands. In several areas ductal epithelium was found surrounded by lymphoid tissue within the developing parotid glands (Fig. 10). This embryo also showed lymphoid tissue developing in the neck region, but no epithelial structures were noted in association with this lymphoid tissue.

The three smaller (crown-rump measurement in mm.) embryos revealed no evidence of developing lymphoid tissue, either in the parotid region or elsewhere in the embryos. (See Table 5) The developing submaxillary and sublingual glands of all the embryos revealed no evidence of lymphoid tissue within them.

Figure 12 shows a portion of the epithelium lining the superior end of the esophagus of the 140 mm. embryo. It is of interest to note that the surface of this epithelium is covered by numerous cilia. Figures 8C, 11, and 13 are examples of some of the lining epithelium found in the embryos.

Fetuses. In twenty-three of the thirty-seven fetuses, lymphoid tissue containing salivary gland epithelium was found within and/or adjacent to the developing parotid glands. (Figs. 17, 19, 20) One fetus revealed a lymph node close to, but not within, the parotid gland.

TABLE 5

THE RELATIONSHIP OF LYMPHOID TISSUE AND DEVELOPING SALIVARY GLANDS IN FOUR EMBRYOS

EMBRYO		DEVELOPING LYMPHOID TISSUE		
Length*	Approx. Age	Parotid	Submaxillary and Sublingual	Neck
140 mm.	16-17 weeks	Present	None	Present
40 mm.	8-10 weeks	None	None	None
37 mm.	8-9 weeks	None	None	None
20 mm.	7-8 weeks	None	None	None

* Measurements represent the crown-rump length.

This lymph node contained apparent salivary gland tissue. (Fig. 21)

Sections of the submaxillary and sublingual glands of the fetuses did not contain lymphoid tissue. Several fetuses, however, revealed lymph nodes close to the submaxillary glands, but these contained no epithelial inclusions. (Fig. 14) None of the lymph nodes in the neck contained epithelial inclusions. The sex, birth weight, age, and pathologic diagnosis of the thirty-seven fetuses is summarized in Table 6.

Radical neck dissections. Seven hundred and ninety-one lymph nodes were examined from the forty-nine cases of radical neck dissections. Only four of these nodes contained salivary gland inclusions and/or small micro-cysts. Each of the nodes came from separate cases and each was from the parotid gland region, with the exception of the one containing a micro-cyst lined by respiratory epithelium. This node was from the submaxillary region. (See Table 7)

Figure 25 reveals a small micro-cyst in a lymph node lined by a respiratory type epithelium. This node was removed from the submaxillary region of a fifty-four year old white male with squamous cell carcinoma of the larynx. Figure 23 shows a small micro-cyst in a lymph node which was lined by an undifferentiated type of epithelium. This node also contained apparent salivary gland tissue. The node was removed from the parotid region of a sixty-eight year old white male with squamous cell carcinoma of the larynx. Figure 24 demonstrates a lymph node with salivary gland tissue within and adjacent to it. This node was removed from the parotid region of a fifty-six year old white male with squamous cell carcinoma of the lower lip. The other positive

TABLE 6 CLINICAL INFORMATION FROM 37 FETUSES

CASE	AUTOPSY NUMBER	SEX	BIRTH WEIGHT	AGE	SIDE OF NECK DISSECTED	PATHOLOGIC DIAGNOSIS
1	60-OA-473	M	2500 gms.	37 wks.	R	Stillborn
2	60-OA-443	M	2510 gms.	36 wks.	L	Atelectasis
3	60-OA-433	M	1160 gms.	29 wks.	L	Prematurity
4	60-OA-409	F	3580 gms.	40 wks.	R	Stillborn
5	60-OA-497	M	1160 gms.		R	Prematurity
6	60-OA-523	M	590 gms.	24 wks.	R	Prematurity
7	60-OA-524	M	560 gms.	24 wks.	L	Prematurity
8	60-OA-60	F	2010 gms.	35 wks.	L	Hyaline Membrane
9	61-OA-122	F	1360 gms.	31 wks.	R	Prematurity
10	61-OA-145	M	1280 gms.	24 wks.	L	Prematurity
11	61-OA-36	F	860 gms.	28 wks.	R	Atelectasis
12	61-OA-144	M	750 gms.	26 wks.	L	Atelectasis
13	61-OA-153	F	610 gms.	24 wks.	R	Prematurity
14	61-OA-163	M	5lb.11oz.		R	Pneumonia
15	61-OA-176	M		Full Tm.	R	Pneumonia
16	61-OA-212	F	1290 gms.	25 wks.	L	Prematurity
17	61-OA-247	M	1150 gms.	28 wks.	L	Prematurity
18	61-OA-260	M	610 gms.	24 wks.	R	Prematurity
19	61-OA-279	F	220 gms.	19 wks.	L	Abortus
20	62-OA-70	M	660 gms.		L	Prematurity
21	62-OA-26	M	1660 gms.	34 wks.	R	Prematurity
22	62-OA-392	M	550 gms.	24 wks.	L	Prematurity
23	61-OA-168	M	710 gms.		L	Prematurity
24	62-OA-71	M	950 gms.	30 wks.	L	Prematurity
25	62-OA-113	F	2200 gms.	35 wks.	L	Stillborn
26	62-OA-104	M		27 wks.	R	Prematurity
27	62-OA-90	F	3590 gms.	36 wks.	R	Stillborn
28	61-OA-417	F	3830 gms.	36 wks.	L	Congenital Heart Disease
29	62-OA-172	M	2110 gms.	36 wks.	R	Prematurity
30	62-OA-214	F	950 gms.		L	Prematurity
31	62-OA-152	M	2150 gms.		R	Stillborn
32	61-OA-543	M	660 gms.	28 wks.	L	Prematurity
33	61-OA-544	M	680 gms.	28 wks.	R	Prematurity
34	62-OA-229	F	1470 gms.	32 wks.	L	Stillborn
35	62-OA-195	M		29 wks.	R	Prematurity
36	61-OA-453	F	700 gms.	24 wks.	R	Prematurity
37	62-OA-405	F	540 gms.	24 wks.	R	Prematurity

TABLE 7

FOUR LYMPH NODES WITH BENIGN EPITHELIAL INCLUSIONS
FOUND AMONG 791 LYMPH NODES FROM RADICAL NECK DISSECTIONS

PATIENT INFORMATION					
Age	Sex	Location and Type of Primary Malignancy	LOCATION OF LYMPH NODE	TYPE OF BENIGN EPITHELIUM FOUND	MUCICARMINE REACTION ON AC NI OR DUCTS
56	M	Squamous cell carcinoma of the lower lip	Parotid Region	Apparent salivary Gland ductal and Acinar epithelium	Negative
54	M	Squamous cell carcinoma of the larynx	Submaxillary Region	Micro-cyst lined by Respiratory Epithelium	None Present
48	M	Squamous cell carcinoma of the Face	Parotid Region	Apparent Salivary Gland Ductal Epithelium	Negative
68	M	Squamous cell carcinoma of the Larynx	Parotid Region	Micro-cyst lined by Undifferentiated type Epithelium. Plus apparent salivary gland acinar epithelium.	Negative

node was similar to the previous one and was removed from the parotid region of a forty-eight year old white female with squamous cell carcinoma of the face.

Mucicarmine stained sections were prepared on all of the above tissue except the lymph node with the micro-cyst lined by pseudo-stratified ciliated columnar epithelium (lack of tissue prevented this extra procedure). The other three nodes were negative for the presence of mucin, which would seem to indicate that the glandular material found was serous in nature and may have arisen from the developing parotid gland. The clinical information from forty of the forty-nine cases of radical neck dissections is shown in Table 8. No clinical information was available on nine of the cases.

Twenty-one of the thirty-one submaxillary glands, removed with the radical neck dissections contained no lymph nodes. However, the remaining ten did show a diffuse chronic inflammatory infiltrate consisting of lymphocytes and plasma cells.

Branchial Cysts

Clinical information. The clinical location of the 149 cysts in this study appeared to be consistent with those reported in the literature. Eighty-two were located in the middle neck region, fourteen in the upper neck, seventeen in the lower neck, one in the piriform sinus, and one in the midline. (Fig. 5) The exact location of thirty-two of the cysts could not be determined. Sixty-five were located in the right side of the neck and sixty-four in the left side. (See Table 9)

The age distribution at time of surgery revealed a pattern different from the reports in the literature. (Fig. 4) Most studies

TABLE 8. CLINICAL INFORMATION FROM 40* CASES OF RADICAL NECK DISSECTIONS

CASE	AGE	SEX	DIAGNOSIS	LOCATION OF PRIMARY	LYMPH NODES WITH EPITH.
1	38	F	Squamous Cell Carcinoma	Alveolar Ridge	No
2	68	M	Squamous Cell Carcinoma	Alveolar Ridge	No
3	58	F	Lymphosarcoma		No
4	44	F	Carcinoma of Thyroid		No
5	76	M	Squamous Cell Carcinoma	Alveolar Ridge	No
6	77	M	Squamous Cell Carcinoma	Tonsil	No
7	66	M	Squamous Cell Carcinoma	Larynx	No
8	71	F	Squamous Cell Carcinoma	Cheek	No
9	73	M	Squamous Cell Carcinoma	Larynx	No
10	56	M	Squamous Cell Carcinoma	Larynx	No
11	71	M	Squamous Cell Carcinoma	Tongue	No
12	40	M	Squamous Cell Carcinoma	Larynx	No
13	44	F	Adenoma	Thyroid	No
14	52	F	Melanoma		No
15	47	F	(Parotidectomy) No Diag.		No
16	56	M	Squamous Cell Carcinoma	Gingiva	No
17	58	F	Squamous Cell Carcinoma	Larynx	No
18	62	M	Squamous Cell Carcinoma	Tonsil	No
19	57	M	Squamous Cell Carcinoma	Tongue	No
20	45	F	Hamartoma Parotid		No
21	73	M	Squamous Cell Carcinoma	Parotid	No
22	46	M	Squamous Cell Carcinoma	Tongue	No
23	72	M	Metastatic Carcinoma		No
24	57	F	Lymphoma		No
25	67	M	Squamous Cell Carcinoma	Larynx	No
26	41	M	Actinomycosis		No
27	49	M	Metastatic Carcinoma		No
28	65	M	Metastatic Carcinoma		No
29	60	F	Metastatic Carcinoma		No
30	32	F	Carcinoma	Thyroid	No
31	68	M	Adenoid Cystic Carcinoma		No
32	71	M	Metastatic Carcinoma		No
33	71	M	Squamous Cell Carcinoma	Lip	No
34	37	F	Carcinoma	Thyroid	No
35	31	F	Carcinoma	Thyroid	No
36	70	M	Squamous Cell Carcinoma		No
37	56	M	Cancer	Lip	Yes
38	54	M	Cancer	Larynx	Yes
39	48	M	Cancer	Face	Yes
40	68	M	Cancer	Larynx	Yes

* Clinical information was available on 40 of the 49 cases used in this study.

TABLE 9

CLINICAL LOCATION OF 149 BRANCHIAL CYSTS

LOCATION	RIGHT	LEFT	OTHER OR NO INFORMATION	TOTAL
Upper Neck	7	7		14
Middle Neck	42	40		82
Lower Neck	9	8		17
Piriform Sinus			1	1
Midline			1	1
Side only listed	7	9		16
No Information			18	18
TOTALS	65	64	20	149

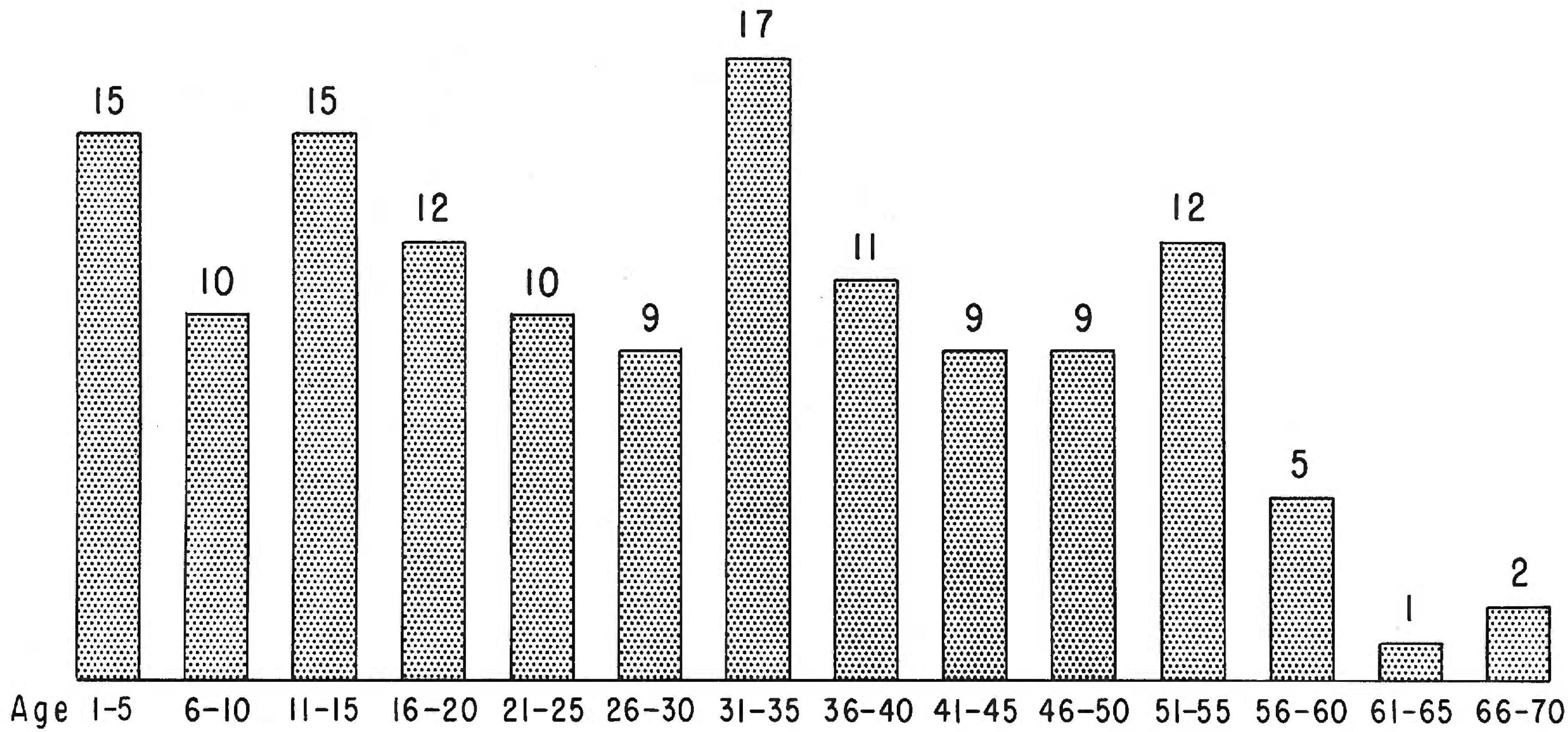


Fig. 4 AGE DISTRIBUTION OF 137 PATIENTS WITH BRANCHIAL CYSTS

report the greatest incidence between ages twenty and forty, but this group shows a relatively equal distribution up to about sixty years of age. The mean age was 28.86 years, median age 29.00 years, and the mode was 15.00 years of age.

Thirty-three cysts had associated sinuses; twenty of which were from patients one to twenty years of age. Ten were from patients twenty-one to forty years of age. Three were from patients forty-one to sixty years of age.

The sex distribution was almost equal, there being seventy females and sixty-five males. Eleven cases did not contain this information. Ninety cases were Caucasion, one Negroid, and no information as to race was available on the other fifty-nine cases.

No one symptom appeared to be present in a significant number of the 138 cases with complete clinical histories other than swelling, (one hundred thirty-four) pain, (twenty-one) and skin drainage. (twenty-six) Four patients described an increase in the size of the lesion during respiratory infections, nine had difficulty in swallowing, and eight patients had recurrent lesions. (See Table 10)

In ninety-six cases it was possible to determine the time elapsed from the initial detection of the lesion (by either patient or professional person) to its surgical removal. The mean elapsed time was 23.44 months, median 3.5 months, and the mode, one month.

Microscopic evaluation. The microscopic examination of the lining epithelium showed that one-hundred and five of the 149 cysts were lined entirely by stratified squamous epithelium. The remaining forty-four or 29.5% were lined in part, or in total, by an epithelium other than stratified squamous. (Fig. 7) Of these forty-four cysts, fifteen were

TABLE 10

CLINICAL SYMPTOMS OF 138 PATIENTS
WITH BRANCHIAL CYSTS

SYMPTOMS	NO. OF PATIENTS
Oral Drainage	0
Skin Drainage	26
Pain	21
Lesion Appeared Infected	20
Fluctuation in Size with Respiratory Infections	4
Recurrent Lesion	8
Difficulty in Swallowing	9
Hoarseness	1
Sore Throat	4
Headache	1
Swelling:	134
Constant for Long Periods	21
Fluctuation in Size	26
No Further Information	36
Sudden Increase in Size	14
Slow Increase in Size	37

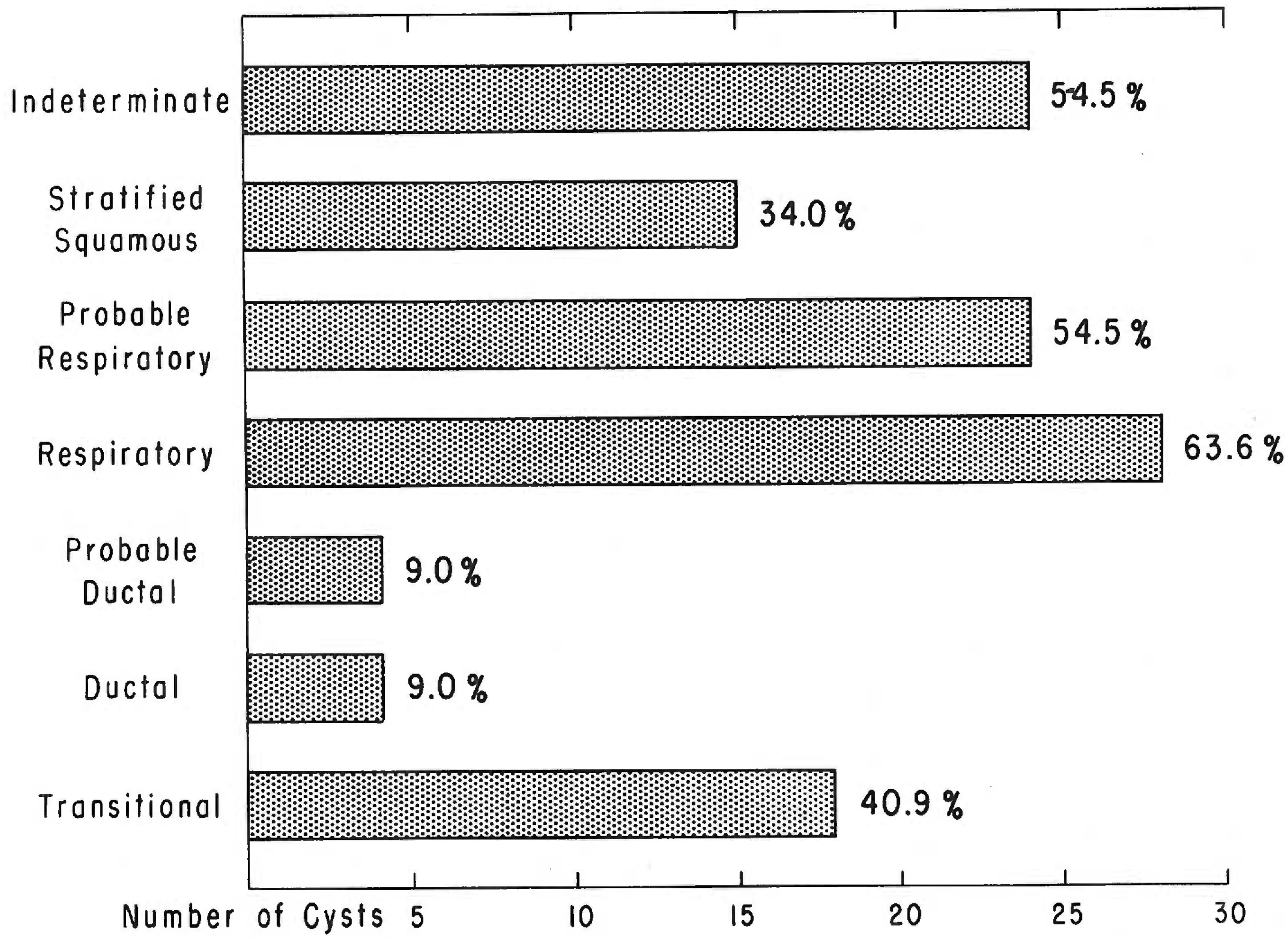


Fig. 7 44 BRANCHIAL CYSTS LINED PARTIALLY OR TOTALLY BY EPITHELIA OTHER THAN STRATIFIED SQUAMOUS*

* Some of these have more than one type of epithelium. Thus, the sum of the bars is greater than 44.

lined in part by stratified squamous, eighteen lined in part by transitional, twenty-six lined in part by respiratory, twenty-four lined in part by probable respiratory, four lined in part by ductal, four lined in part by probable ductal, and twenty-two lined in part by indeterminate epithelium. Two of these cysts were lined entirely by respiratory epithelium and two were lined entirely by an indeterminate type epithelium. Thus, forty cysts were lined by a mixture of epithelial types, and four were lined entirely by epithelium other than stratified squamous.

The total length of lining epithelium was 1,598.8 cm. (Fig. 6) Stratified squamous epithelium constituted 80.3% (1,287.3 cm.) of the total, respiratory 7.6%,(122.0 cm.) probable respiratory 3.7%, (60.5 cm) ductal 0.4%, (6.6 cm.) probable ductal 1.0%, (16.7 cm.) indeterminate 4.6%, (74.7 cm.) and transitional 2.1% (31.0 cm.).

The majority of the cysts were lined completely by stratified squamous epithelium, (70.4%) and the majority of the measured lining epithelium was stratified squamous (80.3%). However, in an attempt to explain the histogenesis of the branchial cyst, the author was most interested in the group of forty-four cysts which had a mixed epithelial lining (forty) or which were lined completely by an epithelium other than stratified squamous (four).

Fourteen of the branchial cysts were found to contain glandular epithelium in their walls. (See Table 11) In twelve of these cases the glands appeared to be a mucous type, and in two a serous type on Hematoxylin-Eosin sections. Mayer's mucicarmine stain was positive for the twelve cysts with the mucous-appearing glands in their walls and

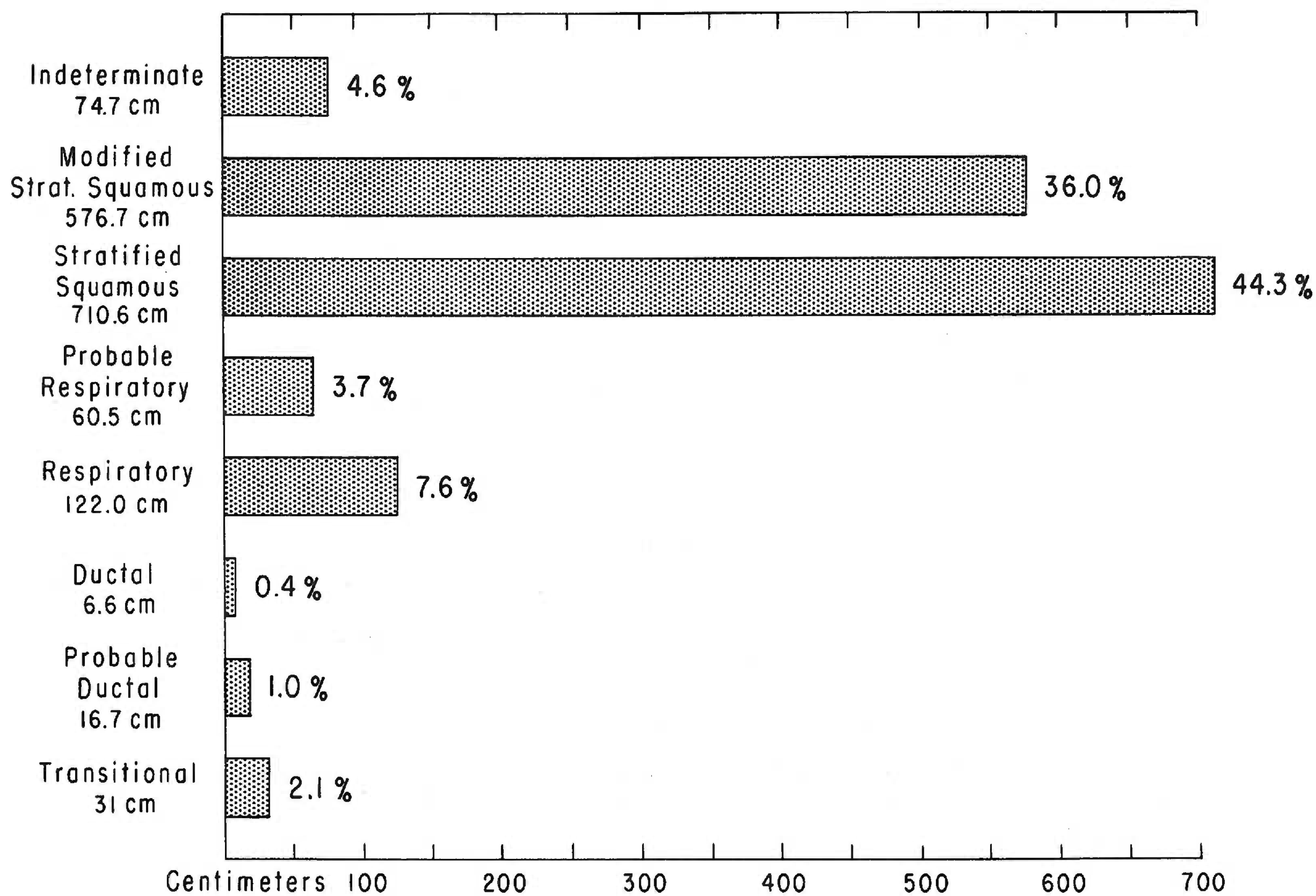


Fig. 6 TOTAL LENGTH IN CENTIMETERS OF THE VARIOUS TYPES OF LINING EPITHELIUM OF 149 BRANCHIAL CYSTS

TABLE 11

FOURTEEN BRANCHIAL CYSTS WITH GLANDULAR EPITHELIUM IN THE CYST WALLS

CASE NO.	MUCICARMINE REACTION OF GLANDS	HISTOLOGY OF GLANDS (H&E)	LINING EPITHELIUM*
15	Positive	Mucous	R, T, PR
51	Positive	Mucous	R, S, PR, I
58	Positive	Mucous	R, PR, I
65	Positive	Mucous	R, T, PR, I
73	Negative	Serous	D, S
84	Positive	Mucous	PR, S, T
103	Positive	Mucous	R
115	Positive	Mucous	R, T, PR, I
153	Positive	Mucous	R, S, T, PR
179	Positive	Mucous	R, S, D, I
183	Negative	Serous	S, D, PD, I
194	Positive	Mucous	R, T
195	Positive	Mucous	R, PR, D, I
198	Positive	Mucous	PD, I

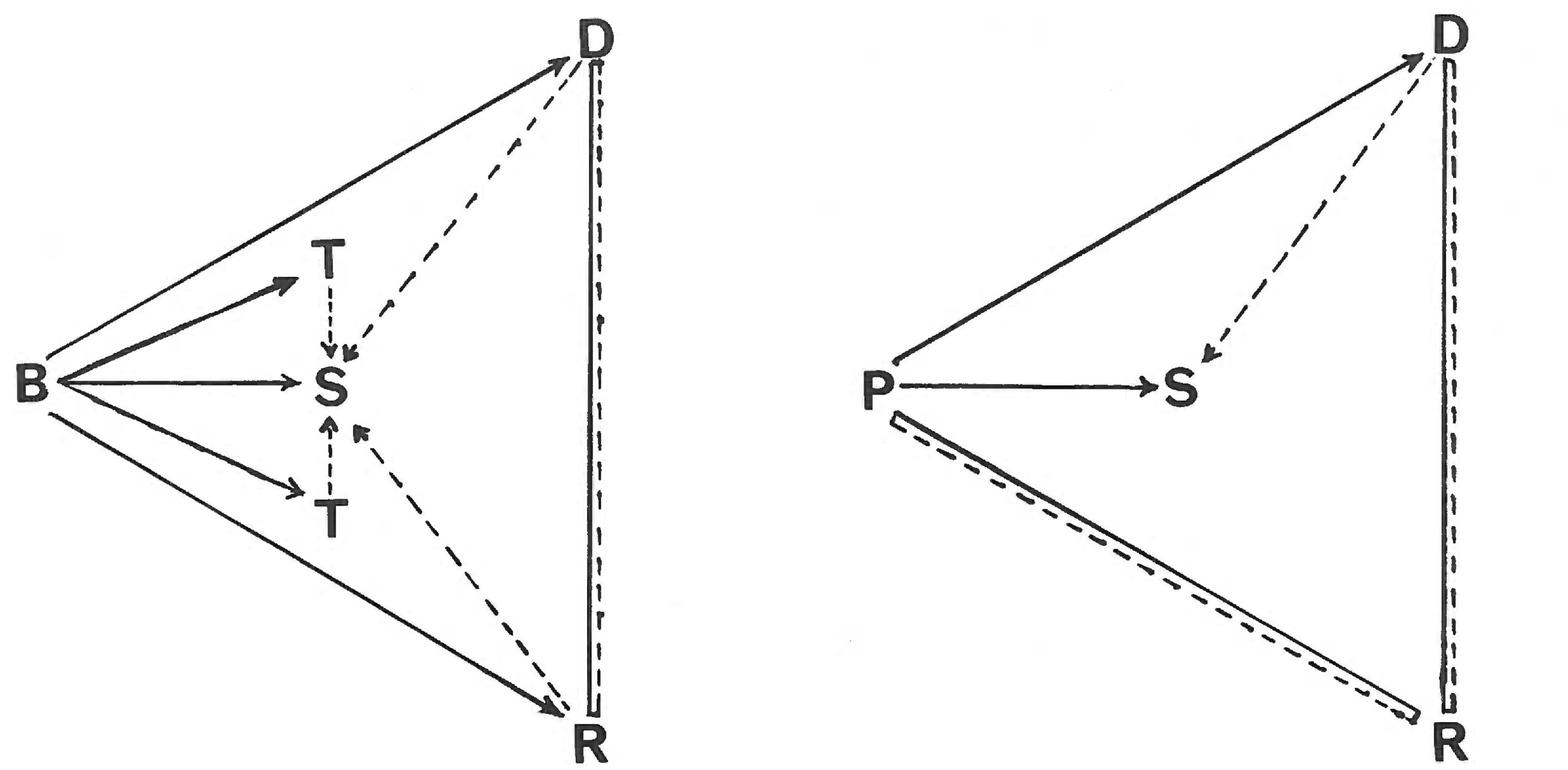
* Abbreviations for the different types of lining epithelium: R - Respiratory, S - Stratified Squamous, T - Transitional, PR - Probable Respiratory, D - Ductal, PD - Probable Ductal, I - Indeterminate.

was negative on the other two cases. Ten of the twelve branchial cysts with mucous glands in their walls were lined in part by respiratory epithelium. The other two were lined by a mixture of epithelia, which did not include respiratory. The two cysts with serous glands in their walls were also lined by a mixture of epithelia, which did not include respiratory epithelium.

The association of epithelium and lymphoid tissue seen in 149 branchial cysts was as follows: Lymphoid tissue in contact with the lining epithelium was noted in 115 of the cysts. Twenty had lymphoid tissue in their walls, but not in contact with the lining epithelium. Fourteen had no lymphoid tissue in their walls.

Figure 3 shows the relationship of the multipotential branchial epithelium and the parotid primordial epithelium. The branchial epithelium may give rise to squamous epithelium, respiratory epithelium, transitional epithelium and ductal epithelium. Once ductal, respiratory or transitional epithelium has been formed, it may, under the influence of environmental stimuli, undergo metaplasia to stratified squamous epithelium. This investigator has been unable to find any reports in the literature which describe ductal epithelium changing to a respiratory type, either in the branchial region or the parotid region.

The parotid primordium may give rise to ductal epithelium, squamous epithelium, and acinar elements (serous). No reports have been noted in the literature describing parotid primordium or parotid gland tissue giving rise to respiratory epithelium. Furthermore, this potential for parotid epithelium (primordial or adult) has not been



BRANCHIAL EPITHELIUM (B) PAROTID PRIMORDIUM (P)

FIG.3 Pathways for differentiation and metaplasia of branchial and parotid epithelium, B - Branchial epithelium, P - Parotid primordium, D - Ductal epithelium, R - Respiratory epithelium, S - Stratified squamous epithelium, T - Transitional epithelium.

- probable routes of development

- probable routes of metaplasia

- routes of metaplasia or development as yet undemonstrated noted by this investigator. Thus, the author would not expect to find any respiratory epithelium in the lining of branchial cysts if they arose from parotid salivary gland tissue entrapped within developing cervical lymph nodes. However, if a significant number of branchial cysts were lined in part or totally by a respiratory epithelium it would seem logical that their origin would not be from parotid epithelium but from an epithelium which had the potential to give rise to respiratory epithelium.

Table 12 represents the twenty-eight cysts which were lined in part or totally by respiratory epithelium. The other types of epithelium found in these cysts are also indicated. Table 13 shows the sixteen cysts lined by a mixture of epithelium (excluding respiratory) or lined totally by an epithelium other than stratified squamous or respiratory. These sixteen were considered to have arisen from parotid epithelium even though only two of them had what is classified as ductal epithelium in their linings. As shown earlier in the paper, these cysts could also have developed from branchial epithelium, but in order to be as conservative as possible they were considered to be of parotid origin.

Statistical analysis. If the hypothesis that branchial cysts develop from parotid gland inclusions in cervical lymph nodes is correct, the number of cysts of parotid origin should be significantly greater than the number of cysts containing respiratory epithelium as part of their lining (non-parotid in origin).

TABLE 12 TWENTY-EIGHT BRANCHIAL CYSTS: TWENTY-SIX LINED BY A MIXTURE OF EPITHELIAL TYPES INCLUDING RESPIRATORY AND TWO LINED TOTALLY BY RESPIRATORY EPITHELIUM

CASE NO.	S	T	R	PR	D	PD	I
15		0.11	0.34	1.95			
28		0.69	7.01				2.30
29			2.07				
41			7.82				4.14
51	1.61		4.37	1.03			1.38
58			2.41	5.40			4.37
65		2.37	2.53	1.95			3.33
67		1.38	14.60	0.69			4.48
70			0.34				0.69
97			3.45	4.60			
103			14.25				
115		1.15	0.80	1.38			1.38
116			0.92	4.60			
118			5.29				2.30
131			0.57	1.72			
138		0.23	1.26	0.69			1.15
142	1.84	2.76	3.91	0.34			0.46
143			4.37				0.46
153	9.31	0.92	1.38	3.45			
162	10.34	9.43	5.75	4.14			0.80
174	11.49	0.34	2.64	4.83			
179	1.95		3.22		2.44		2.76
180		1.07	8.62	4.83			
194		2.99	4.83				
195			3.68	2.99	0.46		0.57
203			7.59	1.26			
206		1.46	6.44	1.49			
207			8.27	1.72			0.23
*Totals	17.54	24.90	122.00	49.06	2.90	0	30.30
Percentages	7.1%	10.1%	49.3%	19.8%	1.2%	0	12.3%

* Amounts of each epithelial type expressed in centimeters.

TABLE 13 SIXTEEN BRANCHIAL CYSTS: FOURTEEN LINED BY A MIXTURE OF EPITHELIAL TYPES EXCLUDING RESPIRATORY AND TWO LINED TOTALLY BY INDETERMINATE EPITHELIUM

CASE NO.	S	T	R	PR	D	PD	I
4	3.45	3.60					
33				8.28			2.87
34	19.20	0.23					
37	10.34	0.34					
53	9.31					0.80	
73	12.87				0.92		
81	8.28	1.15					
84	20.00	0.57		1.15			
85				0.46			2.07
93	2.07			0.34			1.26
136						2.64	0.46
145							33.63
161							2.53
183	10.69				2.83	12.64	0.57
196				1.15			0.57
198						0.57	0.57
*Total	96.21	5.89	0	11.38	3.75	16.65	44.53
Per-cent	53.8%	3.3%	0	6.42%	2.1%	9.3%	25.2%

* Amounts of each epithelial type expressed in centimeters.

1.0 Hypothesis: Number of cysts of ductal origin (parotid) is greater or equal to the number of cysts with respiratory epithelium (not of parotid origin).

2.0 Experimental findings:

2.1 16 cysts considered to be of ductal origin (parotid origin)

2.2 28 cysts with respiratory epithelium (not of parotid origin)

2.3 105 cysts lined entirely by stratified squamous epithelium (no clue as to origin)

3.0 Alpha ≤ 0.05, one-sided test

4.0 Critical region -- less than -1.64

5.0 Test statistic:

5.1 D - parotid origin

R - non-parotid origin

5.2

	x	f	fx	fx^2
D	1	16	16	16
R	0	28	0	0
Total		44	16	16

5.3 Standard deviation ≤ 0.5 (most conservative estimate of deviation in a dichotomy)

5.4 D/D R = 0.3636

5.5 $Z = \frac{.3636-.5}{.07537} = 1.8097$

6.0 -1.8097 falls in the critical region, and therefore the hypothesis is rejected.

By rejecting the hypothesis that branchial cysts develop from parotid salivary gland tissue entrapped in cervical lymph nodes, it was concluded that most branchial cysts do not develop from parotid salivary gland inclusions in cervical lymph nodes. In summary, the statistical analysis consisted of the testing of a null hypothesis, (parotid inclusions give rise to branchial cysts) rejecting it, and therefore accepting the opposite statement (parotid inclusions do not give rise to branchial cysts).

Thyroglossal Duct Cysts

A total of twenty-four cases diagnosed as thyroglossal duct cysts were collected. Eleven of these cysts revealed thyroid tissue in their walls, so only these cases were used in this study as definitely thyroglossal in origin. All eleven cysts were located in the midline. The mean age of the patients was 28.45 and eight of the eleven were males (72%). (See Table 14)

Eight of the eleven cysts were lined, at least in part, by respiratory epithelium. In all cases, the epithelium was positive when stained with Mayer's mucicarmine stain. Four of the eleven had lymphoid tissue in their walls.

TABLE 14

FINDINGS IN 11 CASES OF THYROGLOSSAL DUCT CYSTS WITH THYROID TISSUE IN THE WALL OF EACH CYST

CASE NO.	AGE	SEX	MUCOUS GLANDS IN CYST WALL	LYMPHOID TISSUE IN WALL	EPITHELIAL LINING*
1	13	M	Yes	Yes	S, T
2	1.5	F	No	Yes	R, PD, PR
3	4	M	No	Yes	S, I
4	4	M	No	No	R, PD, I
5	37	M	No	No	R, PD, I
6	78	F	No	No	R, I
7	12	M	Yes	No	R, D, I
8	79	M	No	Yes	S, T, D, PD, I
9	51	M	No	No	R, I
10	10	F	No	No	R, T, S
11	24	M	Yes	Yes	R, T, I

* Abbreviations for the different types of lining epithelium: R - Respiratory, S - Stratified squamous, T - Transitional, PR - Probable Respiratory, D - Ductal, PD - Probable ductal, I - Indeterminate.

DISCUSSION AND CONCLUSIONS

The purpose of this investigation was to examine data which would support or reject the various hypotheses concerning the histogenesis of the branchial cyst, with particular reference to the salivary gland duct inclusion theory.

King (36) and Bernier and Bhaskar (7) have stated that branchial cysts do not arise from remnants of the branchial apparatus for the following reasons:

1. Lesions identical to branchial cysts occur within the parotid gland. These were interpreted as "cystic" lymph nodes.

2. The appearance of fluctuation associated with local inflammation is difficult to explain by the branchial apparatus theory.

3. The presence of lymphoid tissue around these cysts is not accounted for by a branchial origin.

4. The cysts are seldom, if ever, seen at birth.

5. The cysts are connected neither to the pharynx nor to the skin surface.

In contrast to these views, and based on the findings of this study, the following statements are offered:

1. The cysts found in the parotid gland could just as likely develop from branchial remnants as from lymph nodes with salivary gland inclusions. Several cases with a fistula in close association to the parotid gland have been reported. (23) These were thought

to represent remnants of the first branchial groove and/or pouch. It would appear quite possible then that branchial epithelial remnants may be found in the parotid gland, and that these may give rise to the cysts in this location. Even if this was not the case, the mode of origin of the few cysts found within the parotid gland would not necessarily apply to the majority of branchial cysts which are found in other regions of the neck.

2. The follicular (dentigerous) cyst may arise from primitive odontogenic epithelium and become quite large without signs of inflammation, just as a branchial cyst may do. There is no reason why both cannot subsequently become inflamed due to extraneous conditions, as the follicular cyst sometimes does. Those branchial cysts with true lymph node structures may very easily respond to distant sites of inflammation without being caused by them. It should be kept in mind, however, that there are many branchial cysts with no evidence of inflammation either clinically or histologically.

3. A close relationship of epithelium and lymphoid tissue is seen in the eustachian tube, pharyngeal tonsil, adenoids, and the lingual tonsil. In these structures the epithelium appears before the lymphocytes differentiate from the surrounding tissues. (18, 24, 47, 57, 65) These structures develop in part from branchial entoderm. Thus, if the epithelium which gives rise to the branchial cyst were of branchial entodermal origin, it should not be surprising to find an epithelial-lymphoid association developing after the initial epithelial proliferation. Also, it may infrequently be possible that remnants of the branchial apparatus become "enclosed" within developing cervical lymph nodes and later become cystic.

4. This study showed a relatively equal distribution of cysts from birth up to about sixty years of age. (Fig. 4) The mean age was 28.86 years, median age 29.00 years, and the mode was 15.00 years of age.

5. Branchial cysts may be connected with the skin surface if a sinus tract has developed secondary to inflammation within the cyst or if the cyst has been surgically drained.

The branchial cyst, the thyroglossal duct cyst, the follicular (dentigerous) cyst, teratoid cysts and fissural cysts of the maxilla arise from multi-potential epithelial cells and may show a mixture of lining epithelia. Cysts which arise from a more differentiated epithelium, such as radicular and epidermoid inclusion cysts, show a uniform type of epithelial lining. This investigation revealed that the thyroglossal duct cyst may show varying amounts of lymphoid tissue in its wall and may be lined in part by respiratory epithelium. (Table 14) Most investigators agree that this cyst arises from branchial entoderm.

This investigation indicates that the majority of branchial cysts are found in the region between the angle of the mandible and the hyoid bone just anterior to the anterior border of the sternocleido-mastoid muscle. Significantly, salivary gland inclusions in this area were extremely sparse in the tissues from embryos, fetuses, and adults studied. Conversely, parotid salivary gland inclusions in lymph nodes were plentiful in the parotid area, where branchial cysts are rarely seen.

It would seem most logical that the area with the greatest frequency of ductal inclusions in lymph nodes would contain the greatest number of cysts, if the salivary gland inclusion theory were accurate. As mentioned above, this is not the case. However, branchial cysts do arise with the greatest frequency in the area of greatest probable accumulations of epithelial rests from the branchial apparatus.

Several structures found in the human embryo may be the source of the epithelium which lines the branchial cyst. These are the pharyngeal pouches, the cervical sinus, the first branchial groove, and the thymic duct. Those cysts found in the upper neck region could develop from either salivary gland inclusions in lymph nodes or from epithelial remnants of the upper part of the branchial apparatus. Those cysts found in the mid-neck region (below the angle of the mandible to the hyoid bone) could develop from epithelial remnants from the cervical sinus or from the remnants of the branchial pharyngeal pouches. Those cysts found in the lower neck region may develop from remnants of the thymic duct or the cervical sinus.

Burnet (11) suggests lymphocytes or large reticulocytes as a possible origin for the epithelium found in branchial cysts. At the present time this hypothesis must be considered as highly speculative but certainly should encourage further investigation.

Of the branchial cysts in this study, 18.79% contained respiratory epithelium lining at least parts of their lumens. It is difficult to explain the derivation of this respiratory epithelium from parotid duct inclusions in lymph nodes.

The findings of this investigation indicate that most branchial cysts do not develop from parotid salivary gland inclusions in lymph nodes. This conclusion is based on inferences drawn from a statistical analysis of the lining epithelium of 149 branchial cysts. It also appears to be supported by relating the region of the neck which is most likely to contain epithelial remnants to the region of the neck in which the majority of cysts are found.

SUMMARY

1. The histogenesis of the branchial cyst has been a matter of controversy for many years. The most popular hypotheses concerning its origin implicate either the branchial apparatus, the thymic duct, or parotid salivary gland duct inclusions in lymph nodes. The purpose of this investigation was to accumulate data which would tend to support or reject these hypotheses with particular reference to the most recently proposed one, the salivary gland duct inclusion theory. The latter postulates that most branchial cysts take origin from parotid salivary duct tissue trapped in cervical lymph nodes during development.

2. Two avenues of investigation were pursued:

A. Embryos, fetuses, and lymph nodes from adults requiring radical neck dissections were studied to determine the anatomical location of cervical lymph nodes with salivary gland inclusions, and

B. 149 branchial cysts were studied microscopically using Hematoxylin-Eosin and Mayer's mucicarmine stains to identify and quantitate the types of epithelia lining their lumens.

3. Salivary gland inclusions were present in lymph nodes in or adjacent to the parotid gland of embryos, fetuses, and adults. Lymph nodes in other regions of the neck of embryos, fetuses, and adults did not contain salivary gland inclusions.

4. Twenty-three of the thirty-nine fetuses were found to have lymphoid tissue containing salivary gland tissue in or adjacent to the parotid gland.

5. Four lymph nodes of 791 nodes examined from forty-nine radical neck dissections were found to contain benign epithelial tissues; two with micro-cysts and three with apparent salivary gland tissue. Three of these nodes were from the parotid region and the fourth, which was found in the submaxillary region, was lined by respiratory epithelium.

6. Purely on the basis of correlating the area in which branchial cysts occur most frequently with the area of probable greatest concentration of epithelial remains, the branchial apparatus must be considered a more likely source for branchial cysts than the parotid gland.

7. The morphology of the lining epithelia of the floor of the mouth, esophagus, eustachian tube, and trachea in the embryos and fetuses was illustrated.

8. The submaxillary and sublingual salivary glands in the embryos and fetuses studied did not contain lymphoid tissue. Ten of thirty-one submaxillary glands removed as part of radical neck dissections revealed only diffuse infiltrations of mixed chronic inflammatory cells.

9. The lining epithelia of 149 branchial cysts were studied. 120 cysts (80.5%) contained at least some stratified squamous, eighteen (12.0%) transitional; twenty-eight (18.8%) respiratory; twenty-four (16.0%) probable respiratory; four (2.7%) ductal; four (2.7%) probable ductal; and twenty-four (16.0%) contained an indeterminate type of epithelium.

10. The total length of lining epithelium measured from the branchial cysts was 1,598.8 cm. Stratified squamous epithelium constituted 80.3% (1,287.3 cm.) of the total, respiratory 7.6% (122.0 cm.), probable respiratory 3.7% (60.5 cm.), ductal 0.4% (6.6 cm.), probable ductal 1.0% (16.7 cm.), transitional 2.1% (31 cm.), and indeterminate 4.6% (74.7 cm.).

11. The exact origin of the lining epithelium is still not known, but it may originate from various remnants of the branchial apparatus.

12. A statistical analysis of the lining epithelium led to the conclusion that most branchial cysts do not develop from salivary gland inclusions in cervical lymph nodes.

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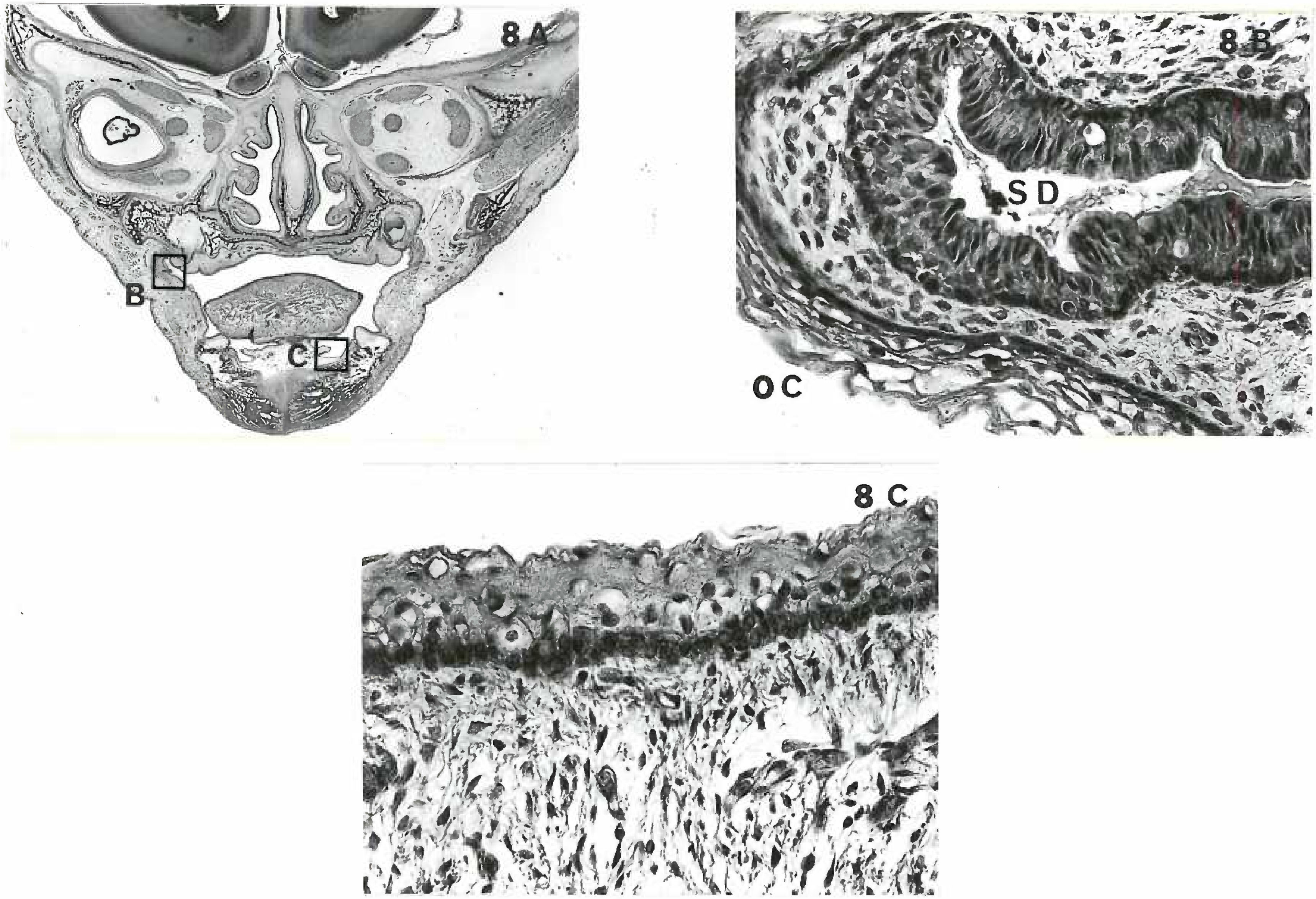
APPENDIX

EMBRYONIC EPITHELIA

Fig. 8A. Frontal section from a 140 mm. embryo (approximate age, 16-17 weeks) at the level of the developing deciduous molars. (H&E, 2X)

Fig. 8B. Higher power view of blocked area (B) in Fig. 8A showing Stenson's duct (SD) near its opening into the oral cavity (OC). Note the ductal epithelium. (H&E, 400X)

Fig. 8C. Higher power view of blocked area (C) in Fig. 8A showing a portion of the epithelium lining the floor of the mouth. Note the basal location of most of the nuclei. (H&E, 400X)



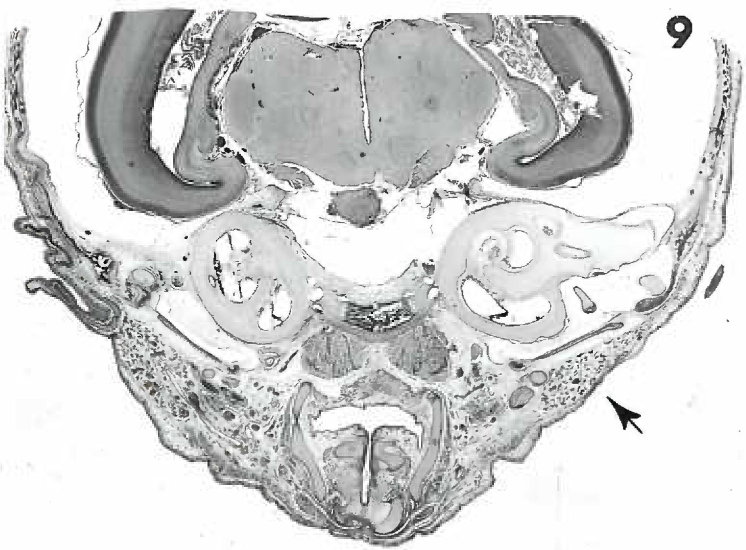
8 A
B
C
8 B
SD
OC
8 C

EMBRYONIC EPITHELIA

Fig. 9. Frontal section from a 140 mm. embryo (approximate age, 16-17 weeks) at the level of the developing parotid glands. The arrow points to a portion of a developing parotid gland. (H&E, 2X)

Fig. 10. Developing parotid gland of a 140 mm. embryo showing ducts in association with lymphocytes. (H&E, 100X)

Fig. 11. Respiratory epithelium from the trachea of a 140 mm. embryo. (H&E, 400X)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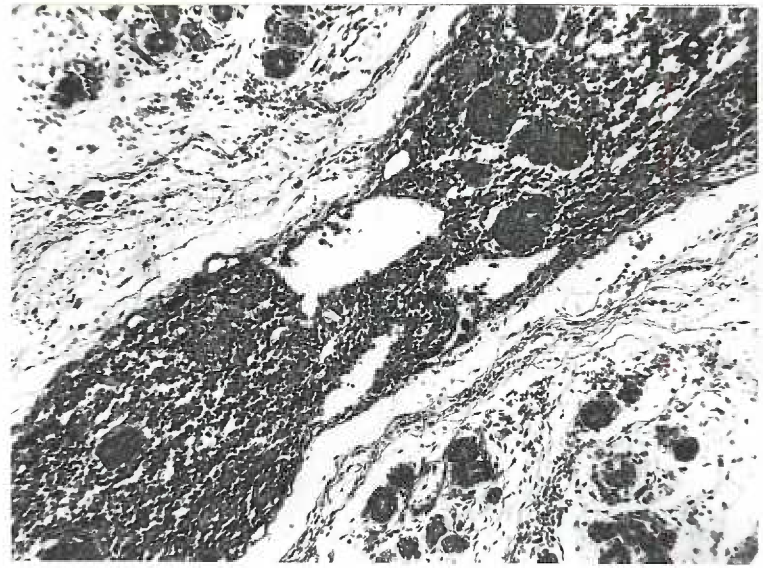


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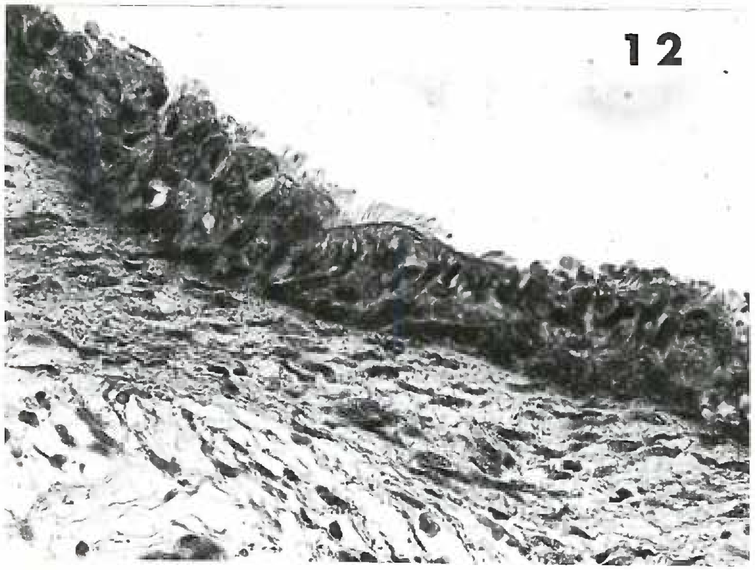
EMBRYONIC EPITHELIA

Fig. 12. Ciliated epithelium from the esophagus of a 140 mm. embryo (approximate age, 16-17 weeks). (H&E, 400X)

Fig. 13. Indeterminate type of epithelium lining the eustachian tube near its opening into the pharynx in a 40 mm. embryo (approximate age, 8-10 weeks). (H&E, 400X)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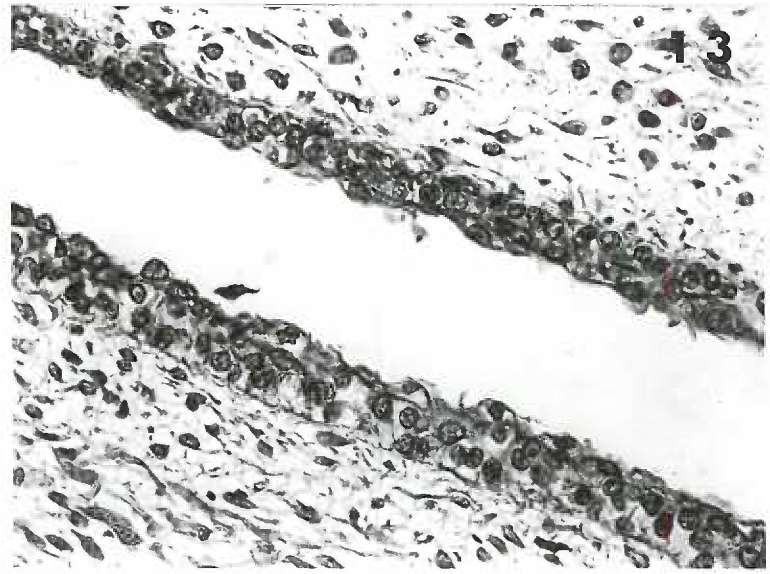
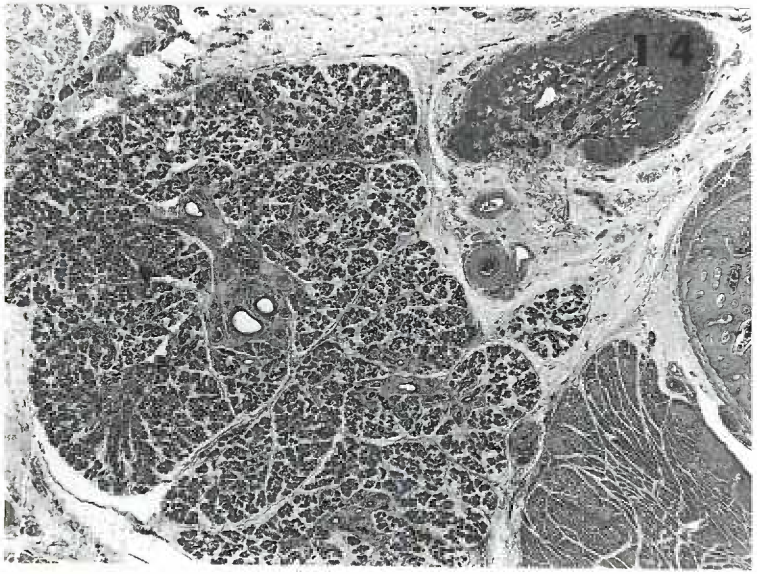

13

FETAL SUBMAXILLARY GLAND AND TONSIL

Fig. 14. Developing submaxillary gland from a fetus (approximate age, 24 weeks). The developing lymph node is near, but not within the gland. Also, note the absence of salivary gland tissue within the lymph node. (Case F36, H&E, 12X)

Fig. 15. Developing tonsil from a fetus (approximate age, 29 weeks). Note the intimate association of lymphoid tissue (L) and epithelium (E). (Case F35, H&E, 12X)

Fig. 16. Developing submaxillary gland from a fetus stained with Mayer's mucicarmine stain. Note the reddish pink staining material within the ducts. This material was interpreted as mucin. (Case F36, H&E, 50X)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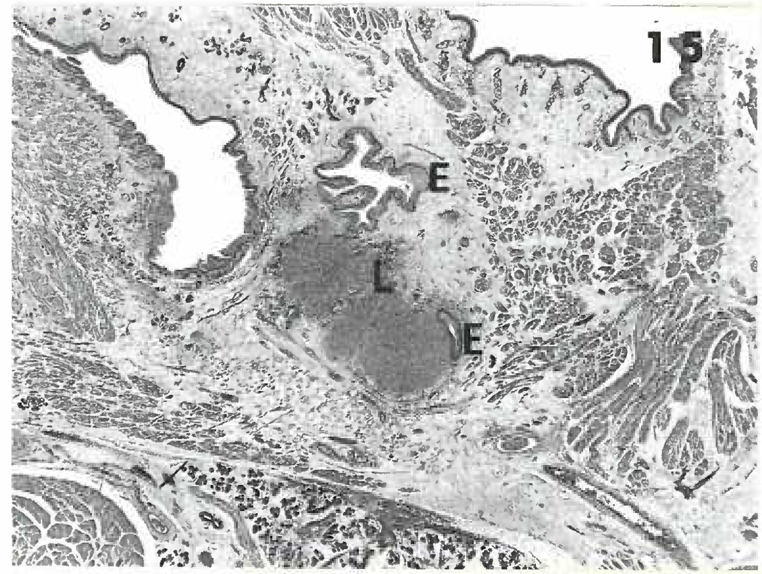
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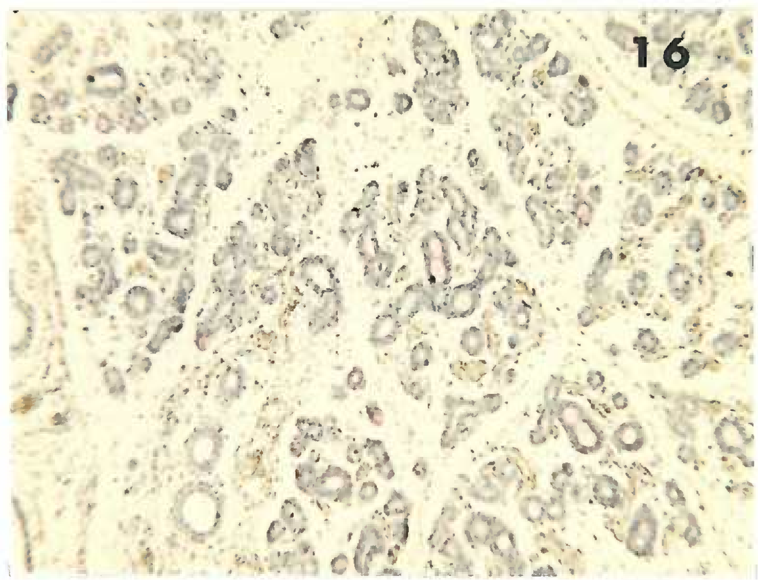
E
L
E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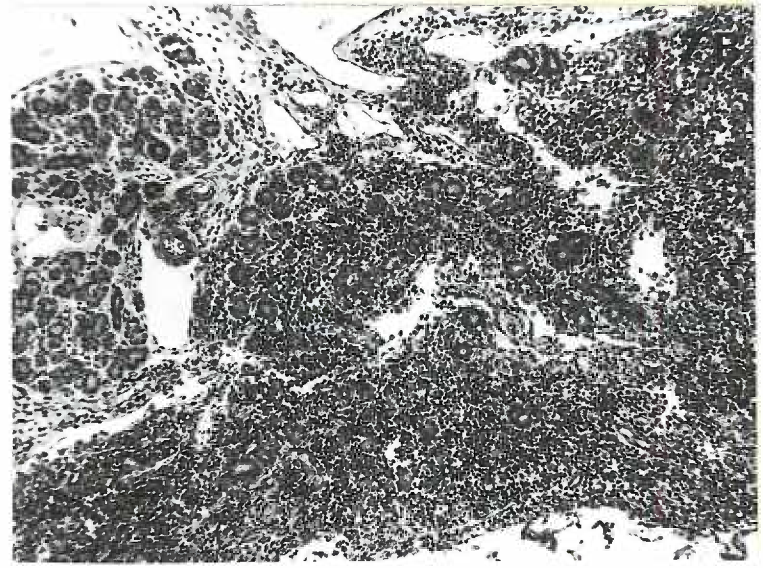
FETAL PAROTID

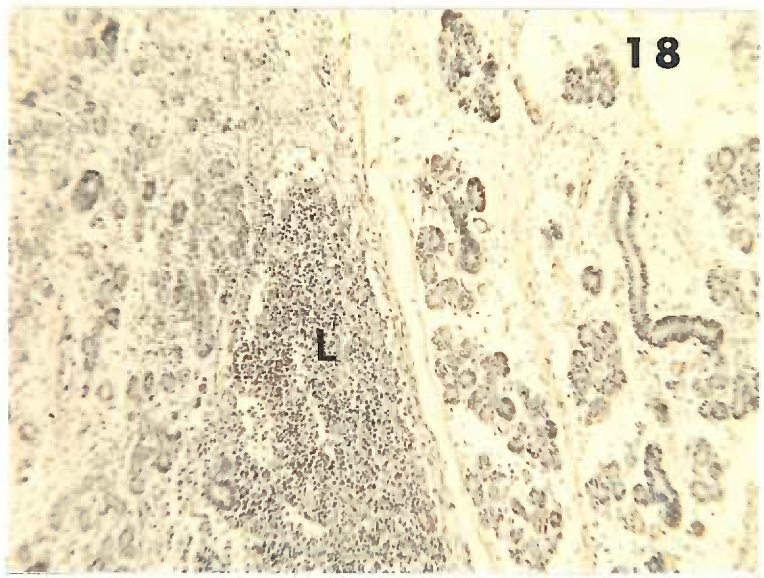
Fig. 17A. Developing parotid gland from a fetus (approximate age, 27 weeks). Note the intermingling of lymphoid and glandular structures (box). (Case F26, H&E, 12X)

Fig. 17B. Higher power view of blocked area in Fig. 17A showing the presence of ductal epithelium within the developing lymphoid tissue. (Case F26, H&E, 100X)

Fig. 18. Developing parotid gland from a fetus stained with Mayer's mucicarmine stain. Note the lymphoid tissue (L). This section was interpreted as negative for the presence of mucin. (Case F26, H&E, 50X)


17 A




18
L

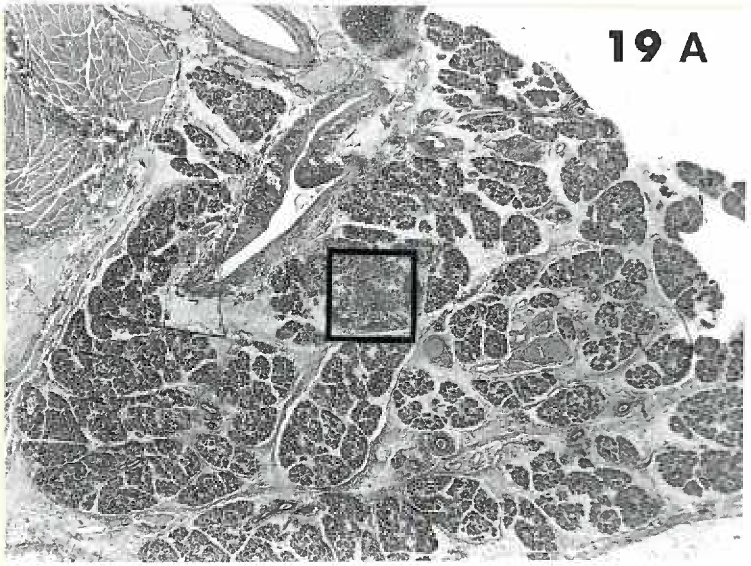
FETAL PAROTID

Fig. 19A. Developing parotid gland from a fetus (approximate age, 37 weeks). Note the intraglandular focus of lymphocytes (blocked). (Case F1, H&E, 12X)

Fig. 19B. Higher power view of blocked area in Fig. 19A. Note the diffuse scattering of lymphocytes which are not organized into a node. (Case F1, H&E, 100X)

Fig. 20A. Developing parotid gland from a fetus (approximate age, 34 weeks). Note the lymphoid tissue within the gland (blocked). (Case F21, H&E, 12X)

Fig. 20B. Higher power view blocked area in Fig. 20A. Note the "cap" of lymphoid tissue above the salivary gland epithelium. (Case F21, H&E, 100X)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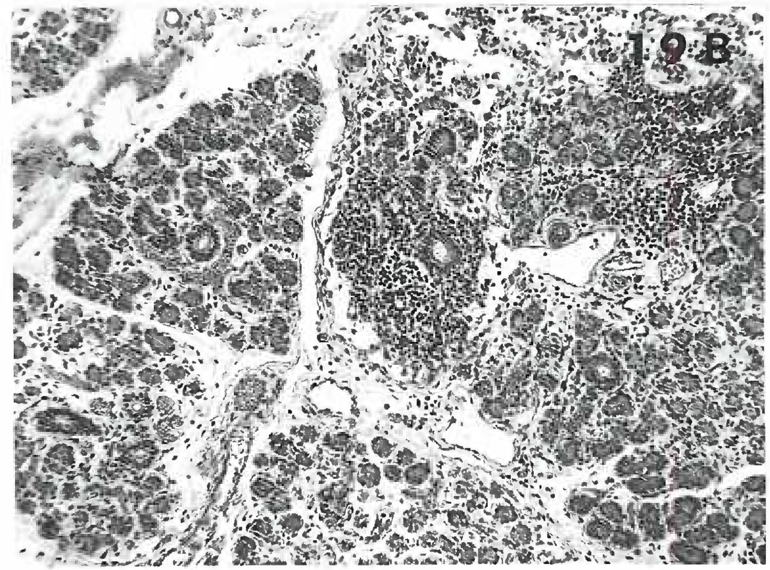
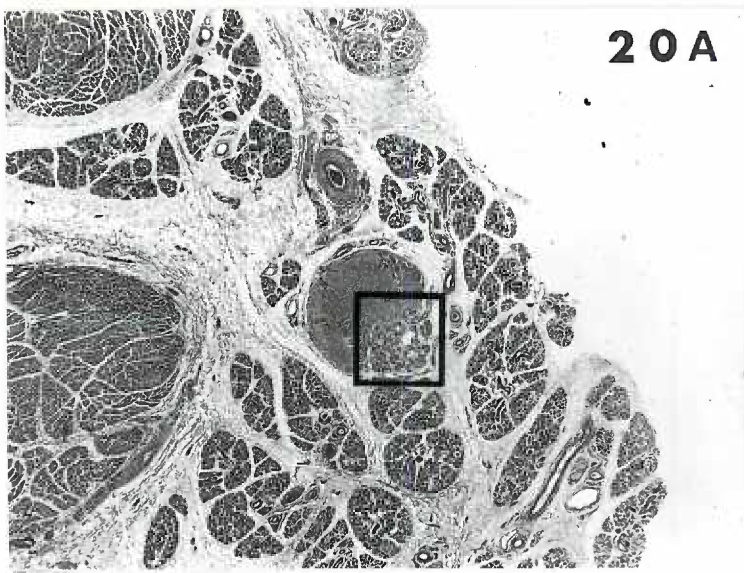
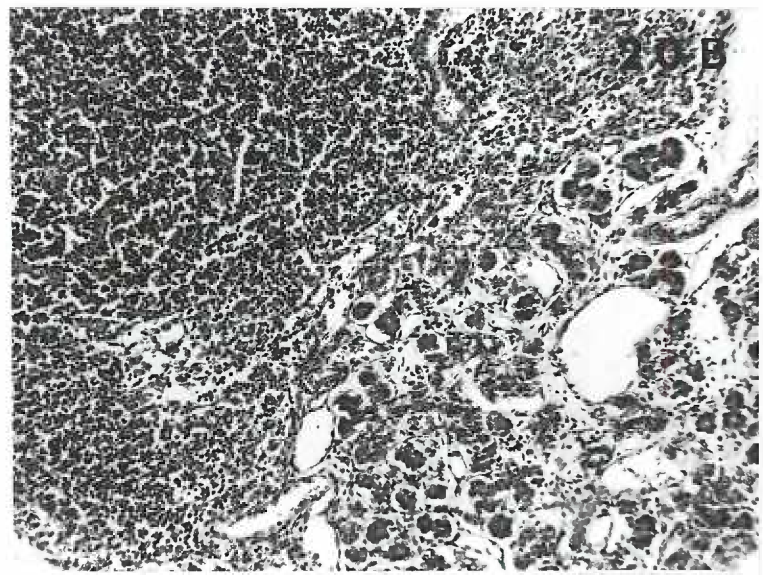

19 B

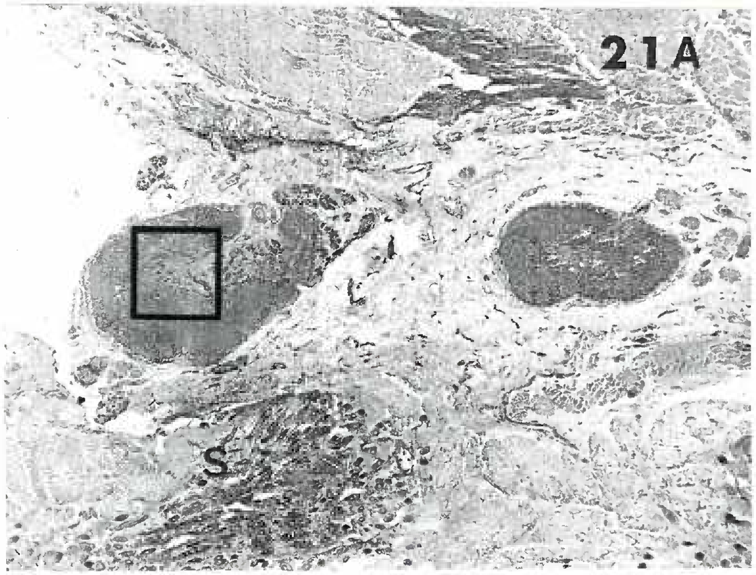

20 A

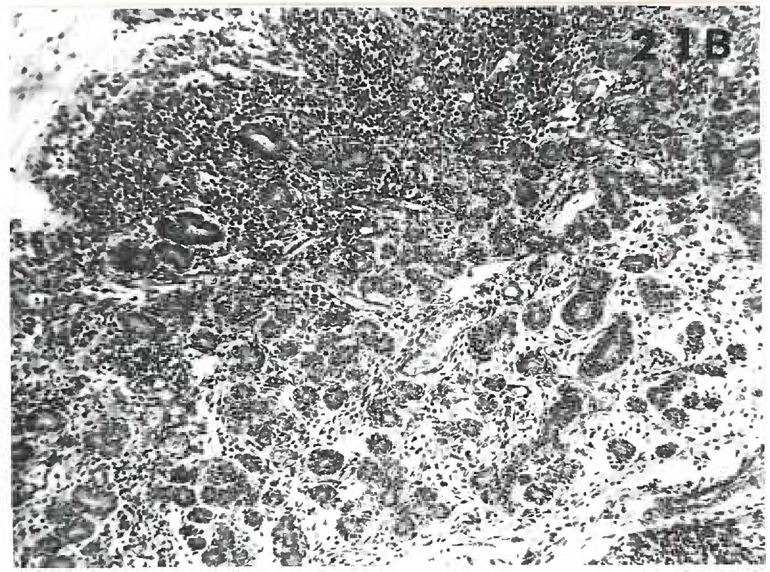

20 B

FETAL PAROTID

Fig. 21A. Developing lymph nodes near the parotid gland of a fetus (approximate age, 24 weeks). Note the oblique section of skin (S) which could be confused with glandular structures at this magnification. (Case F18, H&E, 12X)

Fig. 21B. Higher power view of blocked area in Fig. 21A. Note the ductal structures within the lymph node. (Case F18, H&E, 100X)

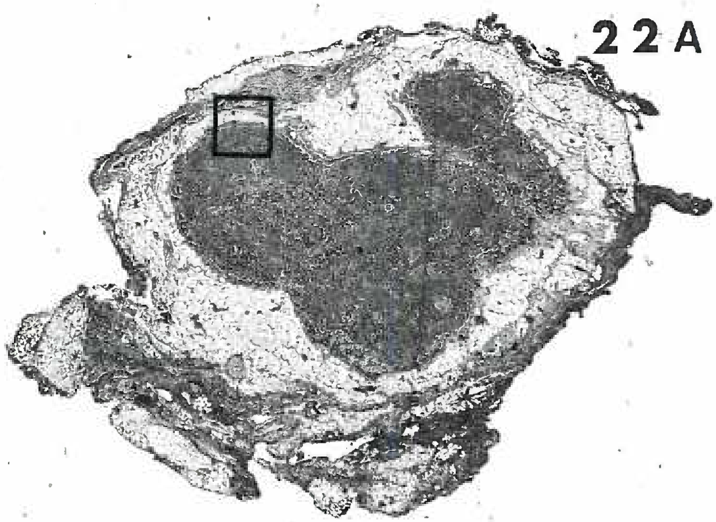

21A
S


21B

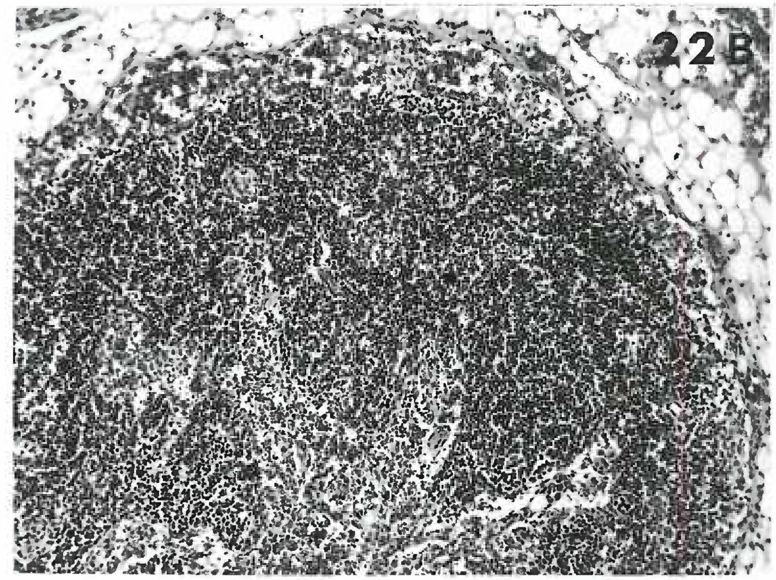
LYMPH NODES FROM RADICAL NECK DISSECTION

Fig. 22A. One of 787 lymph nodes which were removed as part of a radical neck dissection none of which revealed epithelial inclusions. (Case RN18, H&E, 12X)

Fig. 22B. Higher power view of blocked area in Fig. 22A. Note the typical cortical sinusoids. (H&E, 100X)



22A



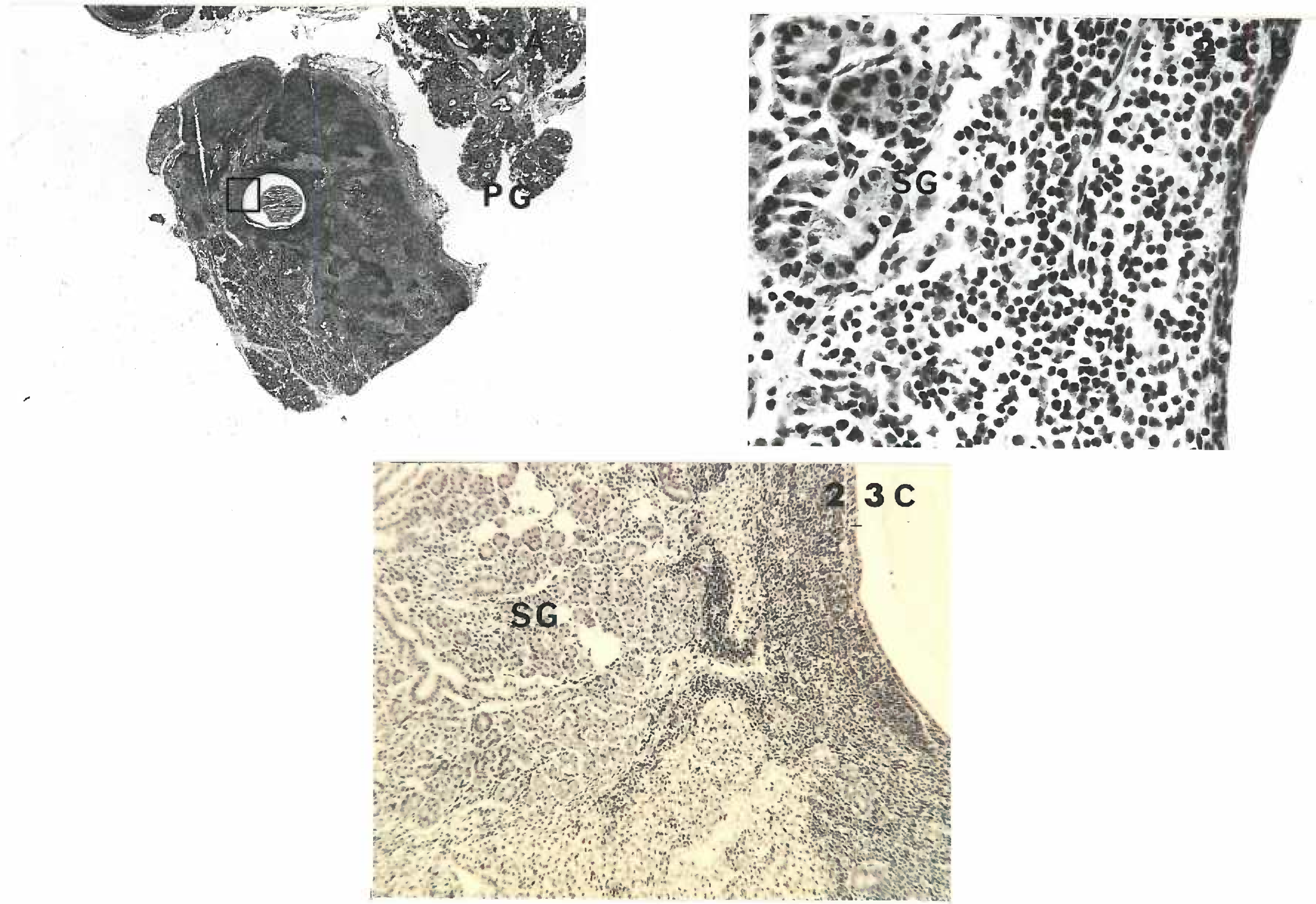
22B

LYMPH NODES FROM RADICAL NECK DISSECTIONS
(ONE OF THE FOUR CONTAINING EPITHELIUM
OUT OF THE TOTAL OF 791 EXAMINED)

Fig. 23A. Lymph node removed from the parotid region during a radical neck dissection. Note the micro-cyst, a portion of which is included in the box. Also, note the section of parotid gland (PG). (Case RN40, H&E, 12X)

Fig. 23B. Higher power view of blocked area in Fig. 23A. Note the apparent salivary gland (SG) epithelium at the right and the indeterminate type of epithelium lining the micro-cyst. (H&E, 400X)

Fig. 23C. Mayer's mucicarmine stain of area shown in Fig. 23B. This was interpreted as negative for the presence of mucin. (200X)


PG
SG
2 3C
SG

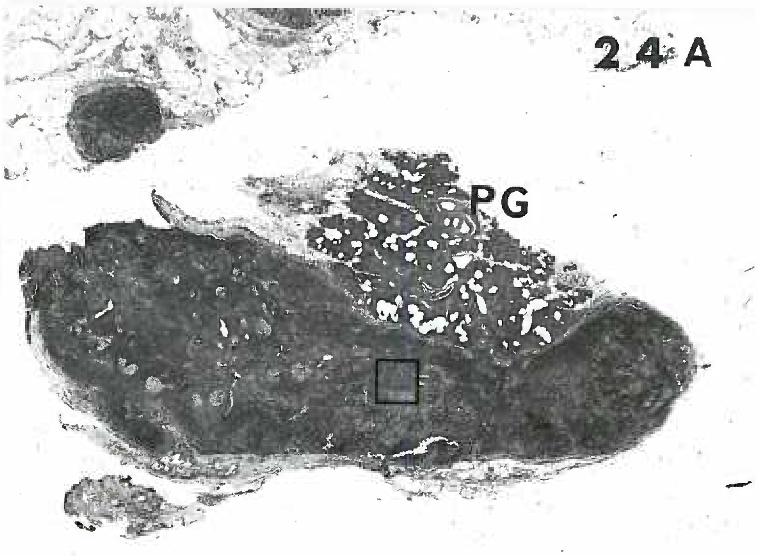
LYMPH NODES FROM RADICAL NECK DISSECTIONS
(TWO OF THE FOUR CONTAINING EPITHELIUM
OUT OF THE 791 EXAMINED)

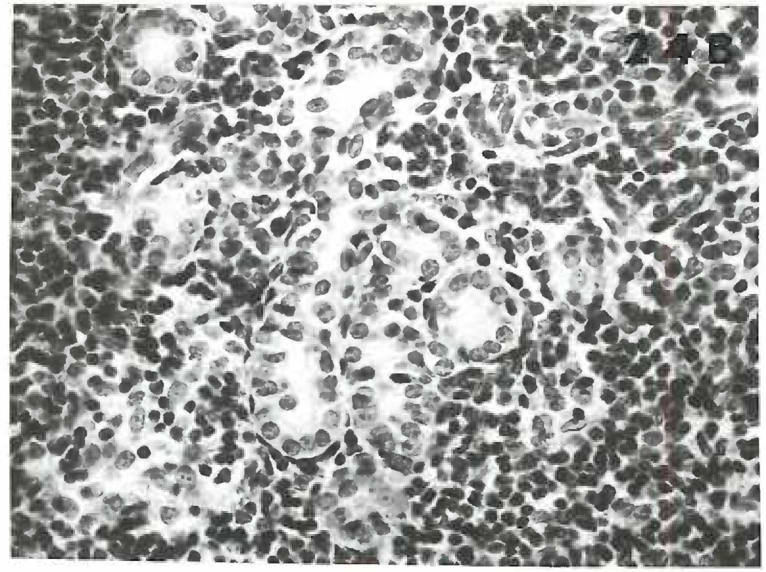
Fig. 24A. Lymph node removed from the parotid region during a radical neck dissection. Note the parotid gland (PG) tissue adjacent to node. (Case RN37, H&E, 12X)

Fig. 24B. Higher power view of blocked area in Fig. 24A. Note the apparent ductal epithelium within the node. (H&E, 400X)

Fig. 25A. Lymph node removed from the submaxillary region during a radical neck dissection. Note the micro-cyst, a portion of which is enclosed in the box. (Case RN38, H&E, 12X)

Fig. 25B. Higher power view of blocked area in Fig. 25A. Note the respiratory type of epithelium lining this cyst. (H&E, 400X)


24 A
PG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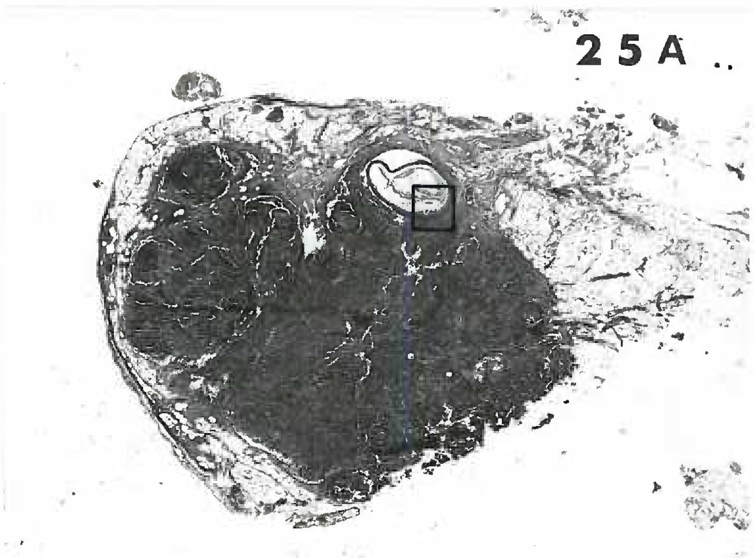
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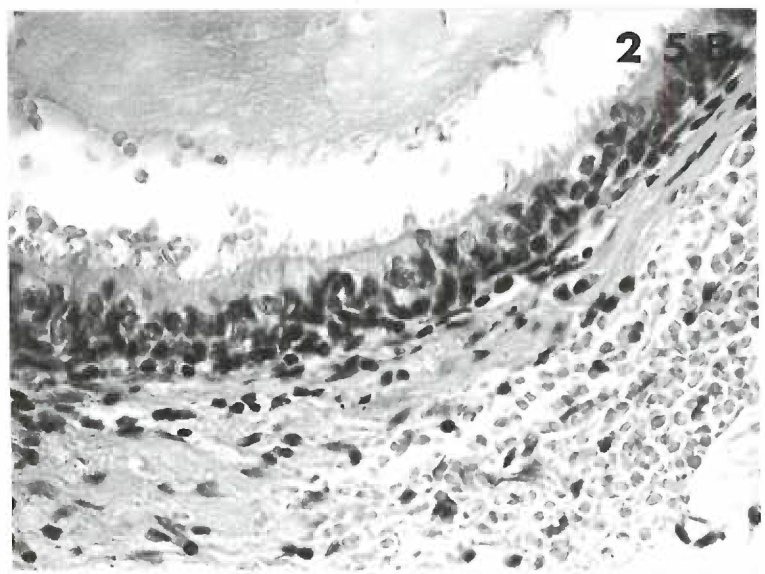

25 B

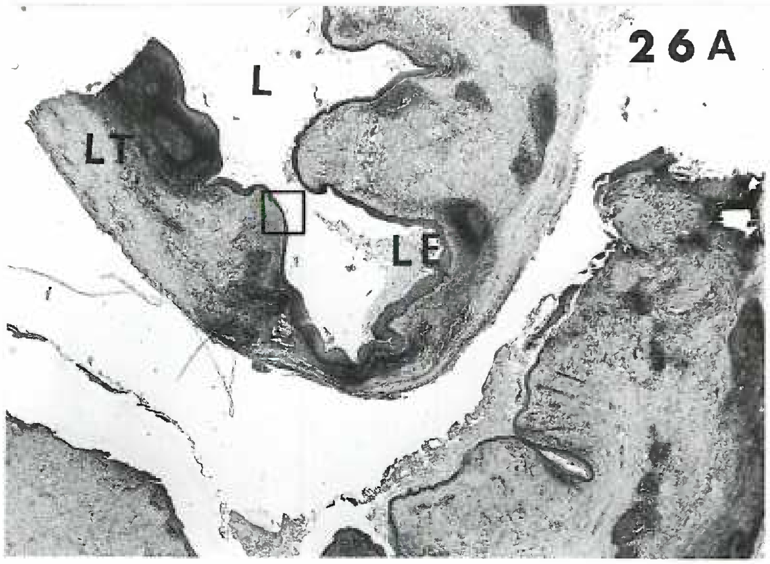
TYPES OF EPITHELIUM LINING BRANCHIAL CYSTS:
STRATIFIED SQUAMOUS AND MODIFIED STRATIFIED SQUAMOUS

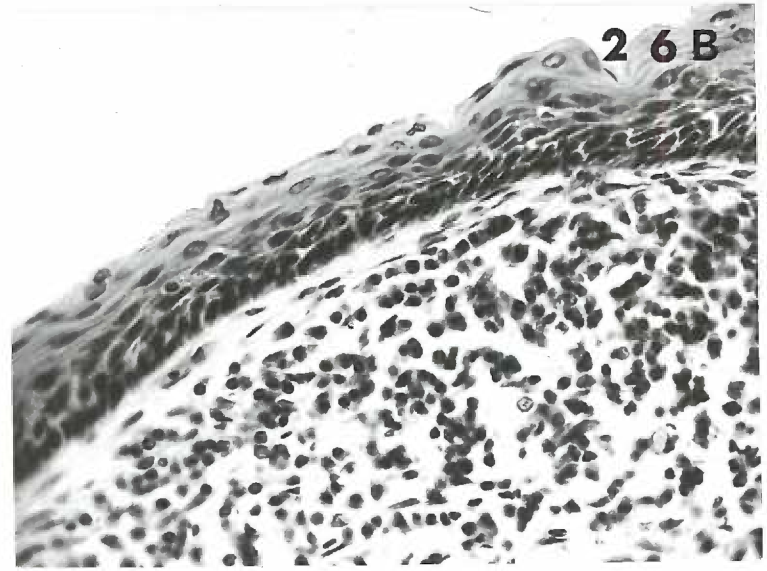
Fig. 26A. A branchial cyst showing the relationship of the lumen (L) lining epithelium (LE), and underlying lymphoid tissue (LT). (Case BC210, H&E, 12X)

Fig. 26B. Higher power view of blocked area in Fig. 26A. This epithelium was classified as stratified squamous. (H&E, 400X)

Fig. 27A. Medium power view of a portion of the lining epithelium of a branchial cyst. Note the underlying lymphoid tissue. This epithelium was classified as modified stratified squamous. (Case BC186, H&E, 100X)

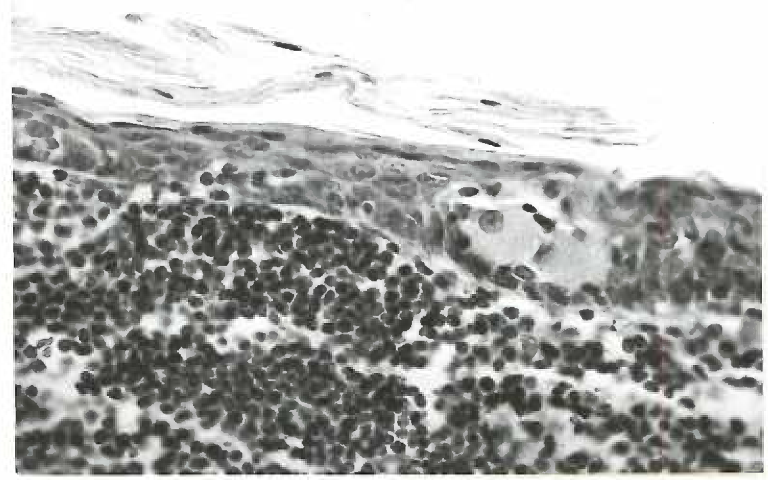
Fig. 27B. High power view of a different portion of the lining epithelium of the cyst shown in Fig. 27A. This epithelium was classified as stratified squamous with keratinization. The mucicarmine stain was negative. (Case BC186, 100X)





27A

27B



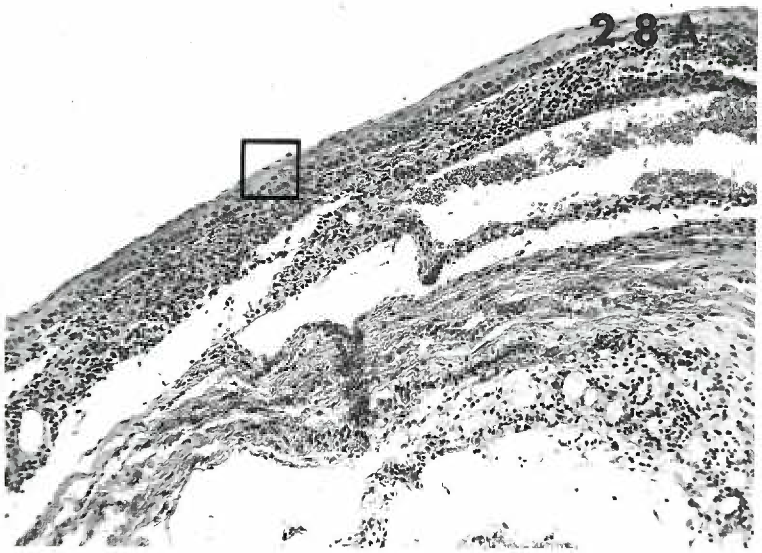
TYPES OF EPITHELIUM LINING BRANCHIAL CYSTS:
PROBABLE DUCTAL. DEVELOPING BRANCHIAL CYSTS

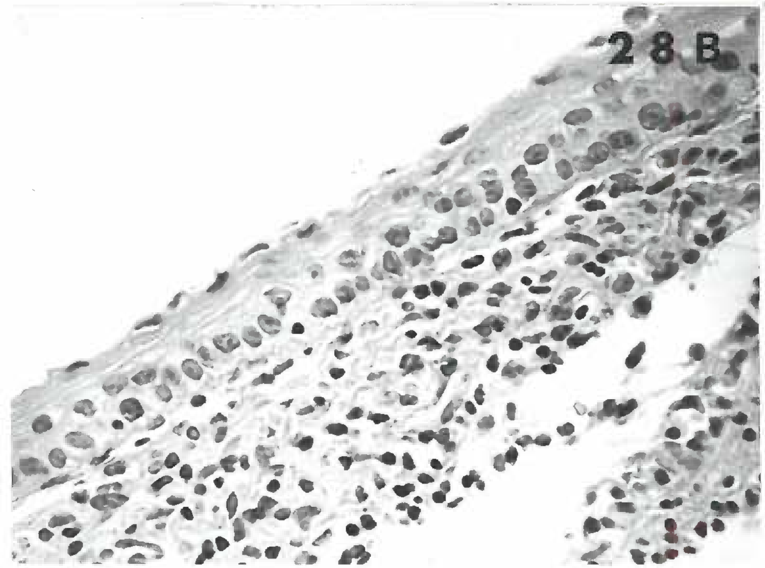
Fig. 28A. Medium power view of a portion of the lining epithelium of a branchial cyst. (Case BC53, H&E, 100X)

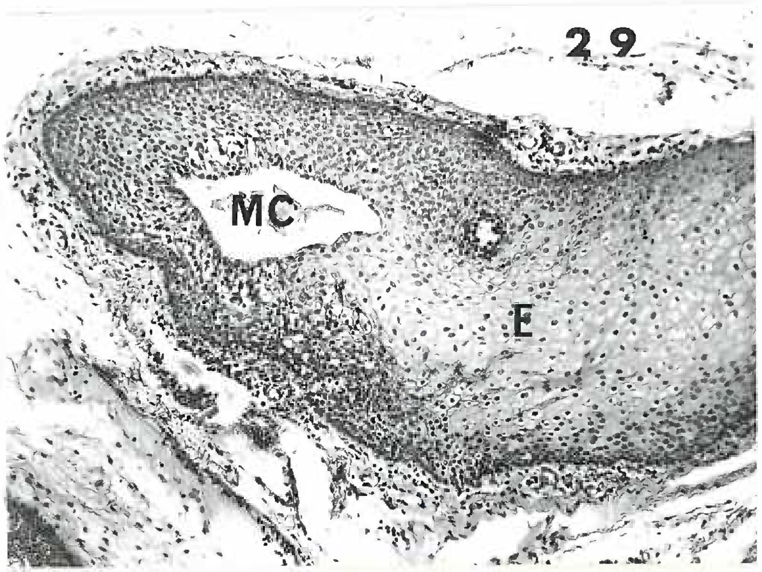
Fig. 28B. Higher power view of blocked area in Fig. 28A. Note the squamoid features at the surface and the single to double row of nuclei at the basal portion. Although this is most likely stratified squamous epithelium, it was placed in the probable ductal category. (Case BC53, H&E, 400X)

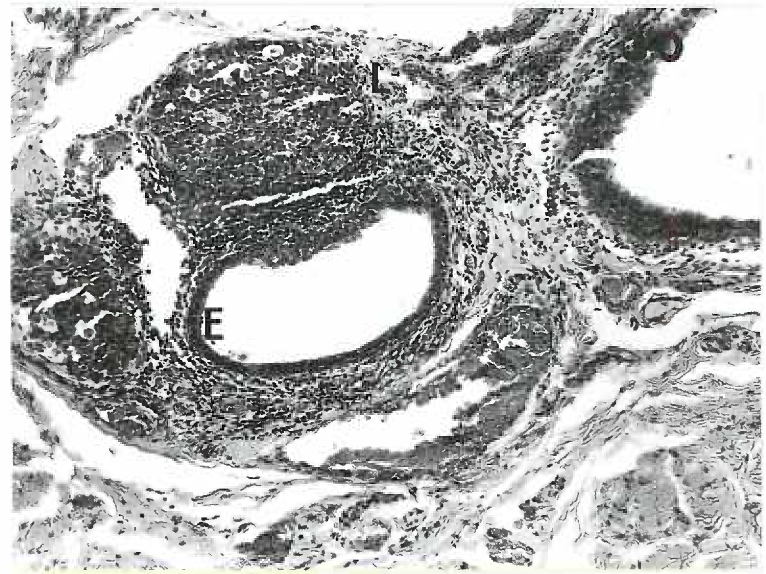
Fig. 29. Micro-cyst (MC) developing in a clump of epithelium (E) located in the fibrous connective tissue wall of a branchial cyst. (Case BC84, H&E, 100X)

Fig. 30. Association of epithelium (E) and lymphoid tissue (LT) noted in a branchial cyst. (Case BC138, H&E, 100X)


28 A


28 B


29
MC
E

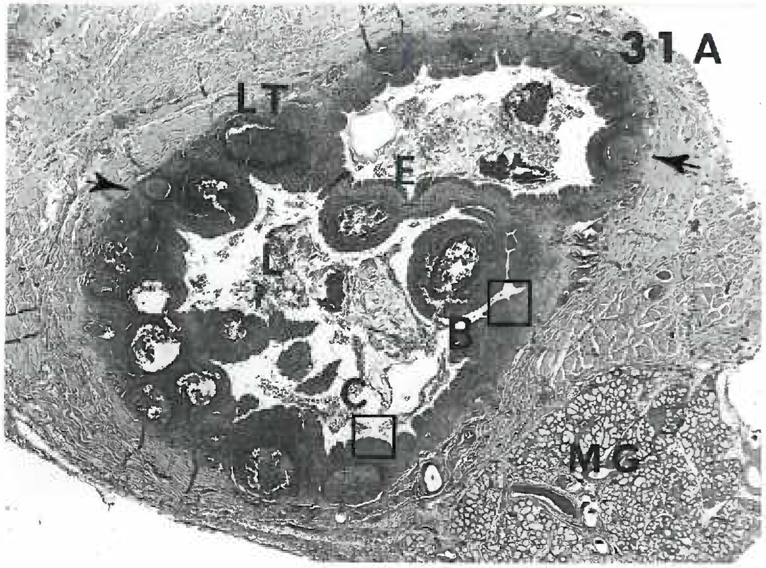

L
E

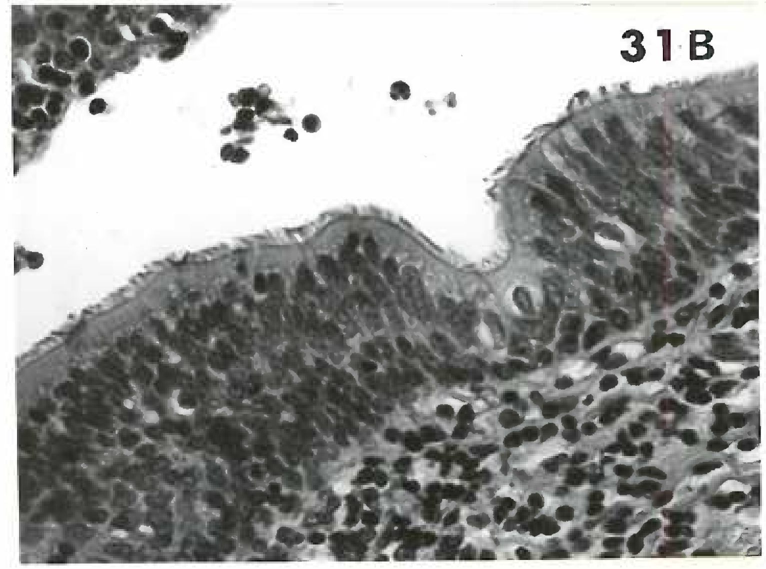
TYPES OF EPITHELIUM LINING BRANCHIAL CYSTS: RESPIRATORY

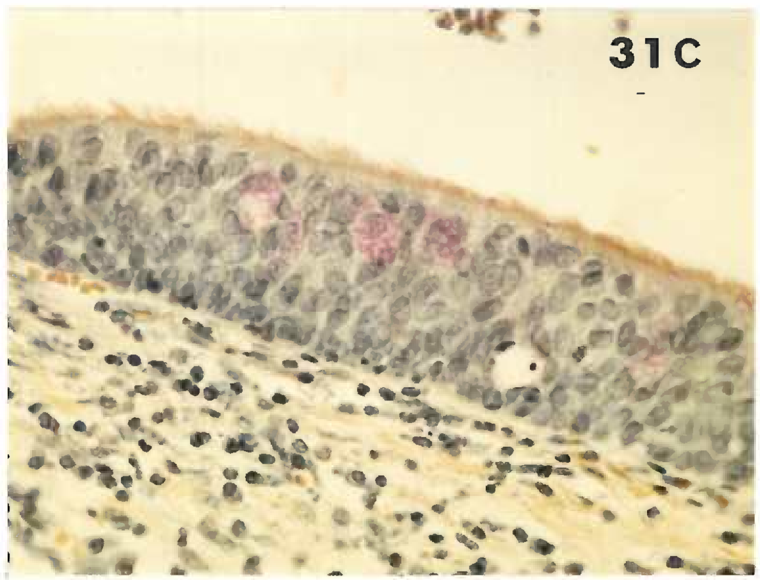
Fig. 31A. A branchial cyst showing the relationship of lumen (L), lymphoid tissue (LT), and the lining epithelium (E). Note the germinal centers (arrows) in the lymphoid tissue and the mucous glands (MG) in the lower right corner. (Case BC51, H&E, 12X)

Fig. 31B. Higher power view of blocked area (B) in Fig. 31A. This epithelium was classified as a respiratory type. (H&E, 400X)

Fig. 31C. Higher power view of blocked area (C) in Fig. 31A. Note the light red material in the epithelium. This material was interpreted as mucin. (Mayer's mucicarmine stain, 400X)


31 A
LT
E
L
B
C
MG


31 B

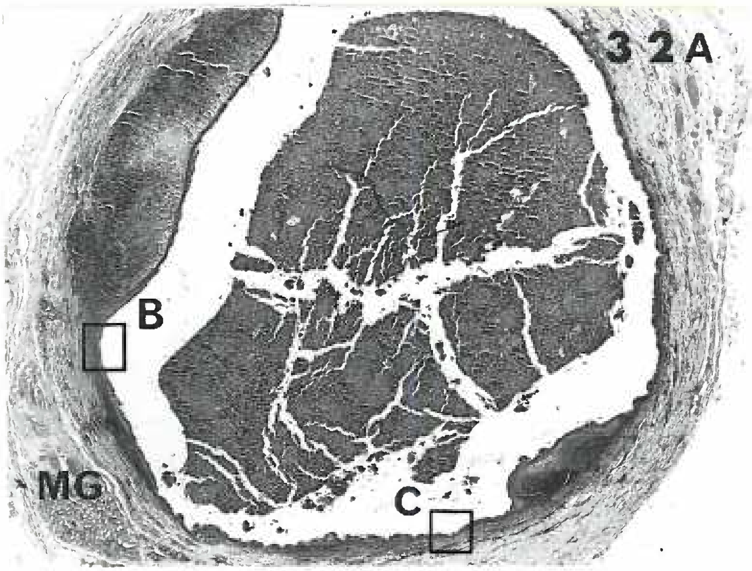

31 C

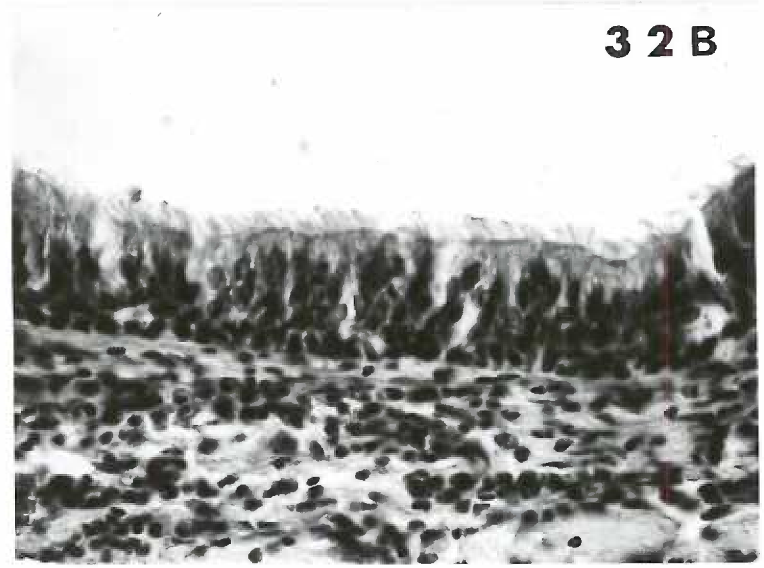
TYPES OF EPITHELIUM LINING BRANCHIAL CYSTS: RESPIRATORY

Fig. 32A. A branchial cyst showing the relationship of lymphoid tissue and the lining epithelium. Note the mucous glands (MG) in the lower left corner. (Case BC67, H&E, 12X)

Fig. 32B. Higher power view of blocked area (B) in Fig. 32A. This epithelium was classified as a respiratory type. (H&E, 400X)

Fig. 32C. Higher power view of blocked area (C) in Fig. 32A. Note the light red material in the respiratory epithelium. This material was interpreted as mucin. (Mayer's mucicarmine stain, 400X)


32A
B
C
MG


32B


32C

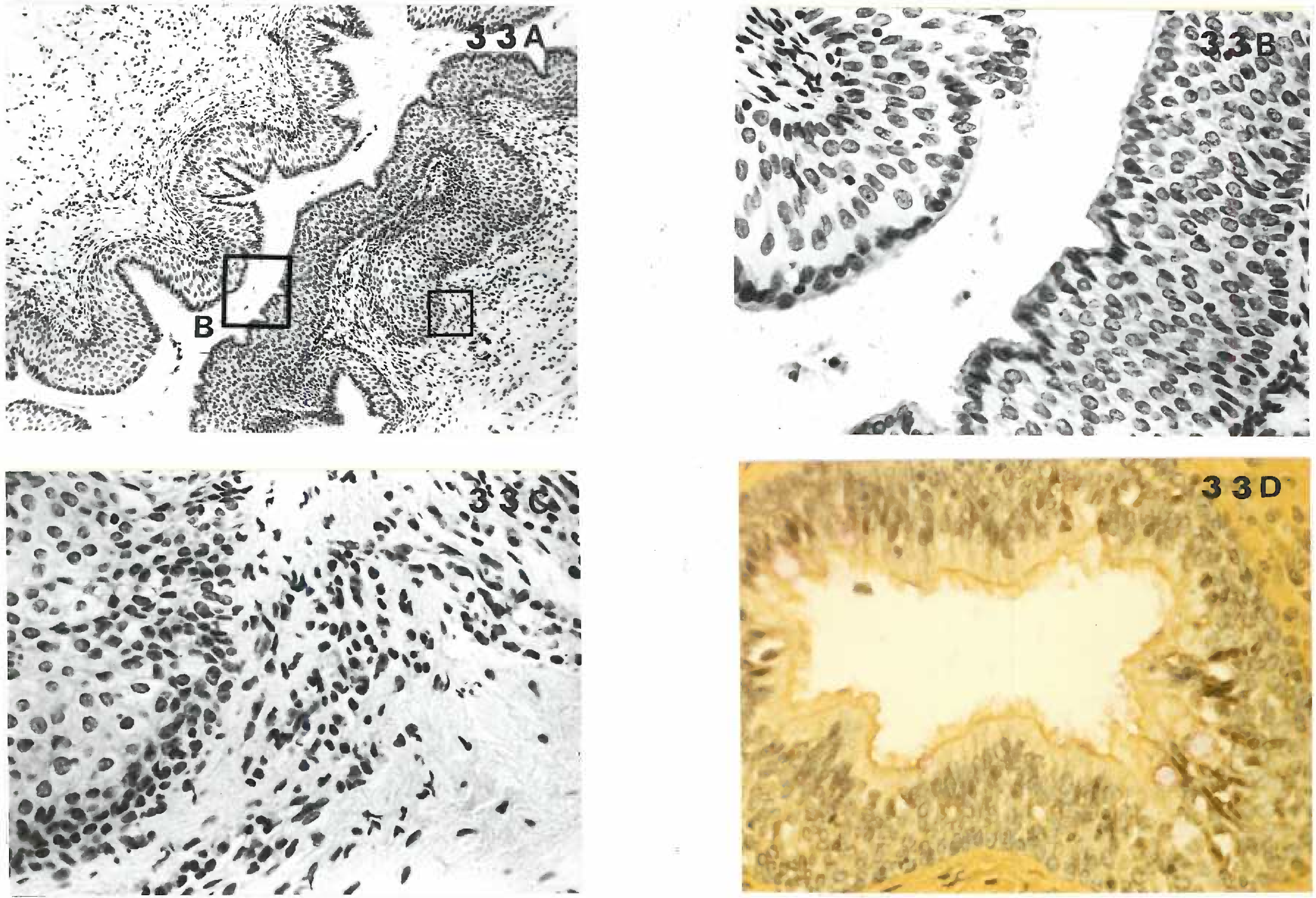
TYPES OF EPITHELIUM LINING BRANCHIAL CYSTS:
TRANSITIONAL AND RESPIRATORY

Fig. 33A. Medium power view of a portion of the epithelium lining a branchial cyst. This was classified as transitional epithelium. Other portions of this cyst were lined by respiratory epithelium. (Case BC194, 100X)

Fig. 33B. Higher power view of blocked area (B) in Fig. 33A. (H&E, 400X)

Fig. 33C. Higher power view of blocked area (C) in Fig. 33A. Note the sparse accumulation of lymphocytes beneath the transitional epithelium. (H&E, 400X)

Fig. 33D. High power view of another portion of the branchial cyst shown in Fig. 33A. Note the light red staining material in the respiratory epithelium. This material was interpreted as mucin. (Mayer's mucicarmine stain, 400X)


33A
B
33B
33C
33D

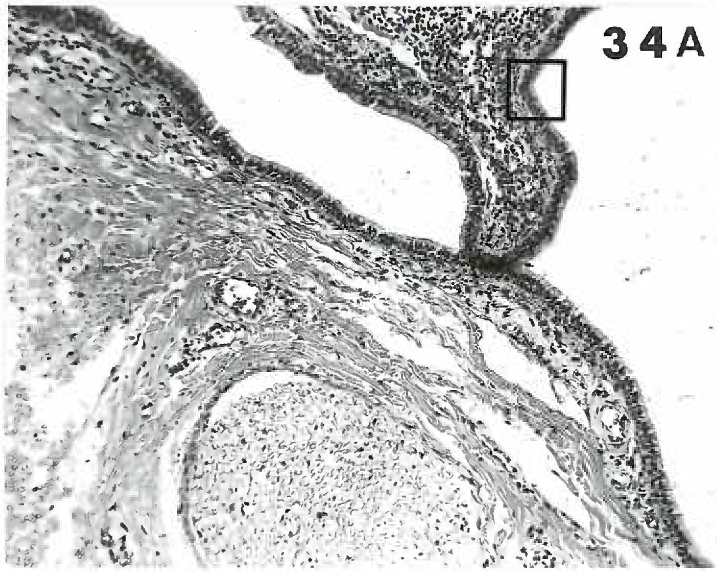
TYPES OF EPITHELIUM LINING BRANCHIAL CYSTS:
DUCTAL AND INDETERMINATE

Fig. 34A. Medium power view of a portion of the lining epithelium of a branchial cyst. (Case BC73, H&E, 100X)

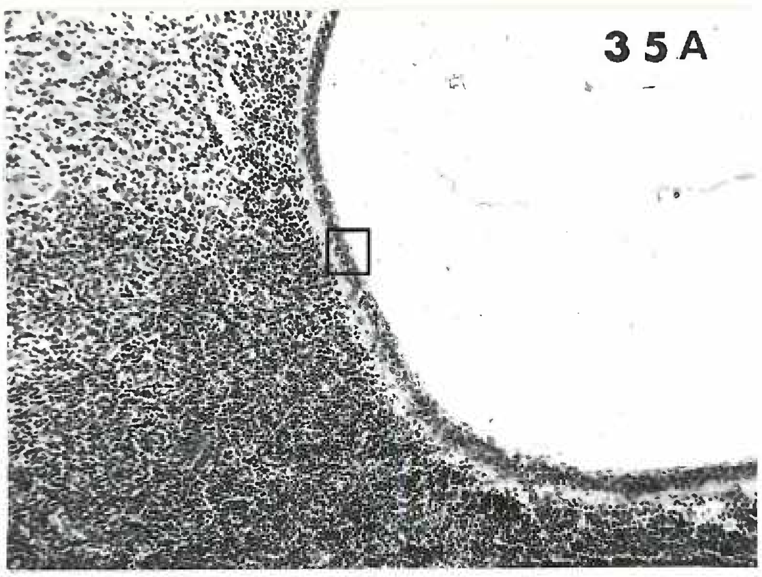
Fig. 34B. Higher power view of blocked area in Fig. 34A. This was classified as ductal epithelium. (H&E, 400X)

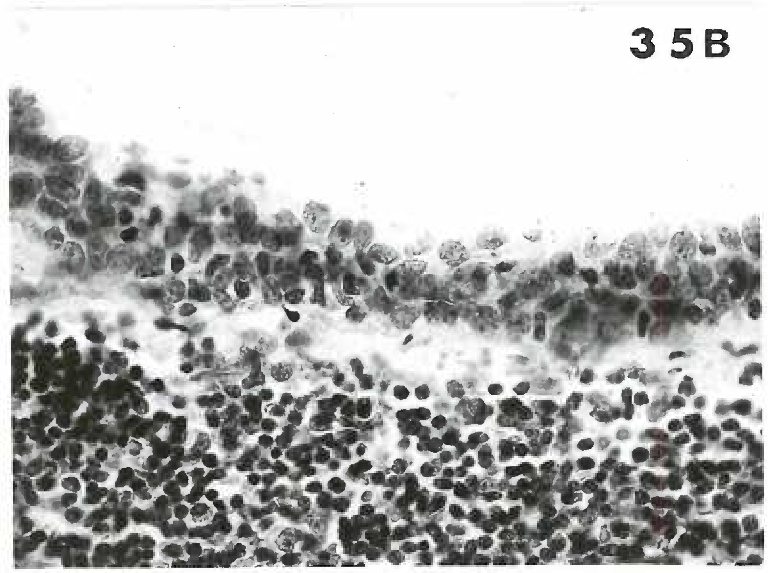
Fig. 35A. Medium power view of a portion of the lining epithelium of a branchial cyst. (Case BC183, H&E, 100X)

Fig. 35B. Higher power view of blocked area in Fig. 35A. This was classified as indeterminate type of epithelium. (H&E, 400X)


34A


34B


35A


35B

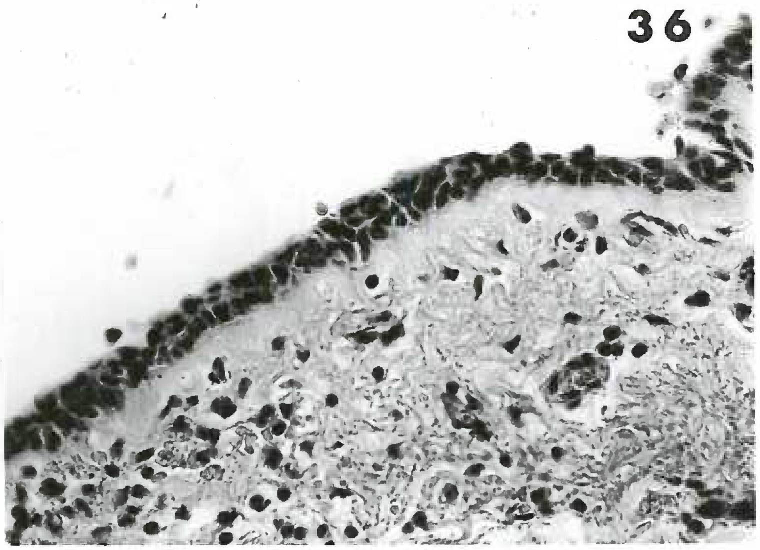
TYPES OF EPITHELIUM LINING BRANCHIAL CYSTS:
INDETERMINATE AND DUCTAL

Fig. 36. High power view of a portion of the lining epithelium of a branchial cyst. This epithelium was classified as indeterminate. (Case 85, H&E, 400X)

Fig. 37. High power view of a portion of the lining epithelium of a branchial cyst. This simple columnar epithelium was classified as indeterminate. (Case BC145, H&E, 400X)

Fig. 38A. Medium power view of a branchial cyst. Note the mucous glands (MG) and dilated ducts (DD) in the fibrous connective tissue wall of this cyst. Most of this cyst was lined by a respiratory type epithelium (not shown in this view). (Case BC195, H&E, 100X)

Fig. 38B. Higher power view of blocked area in Fig. 38A. The structures noted on the surface of the epithelium proved not to be cilia. Epithelium was classified as ductal. (H&E, 400X)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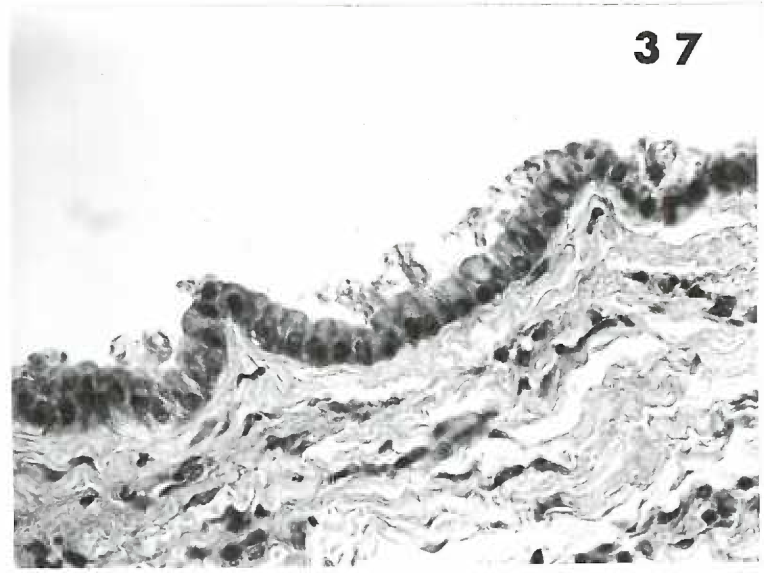
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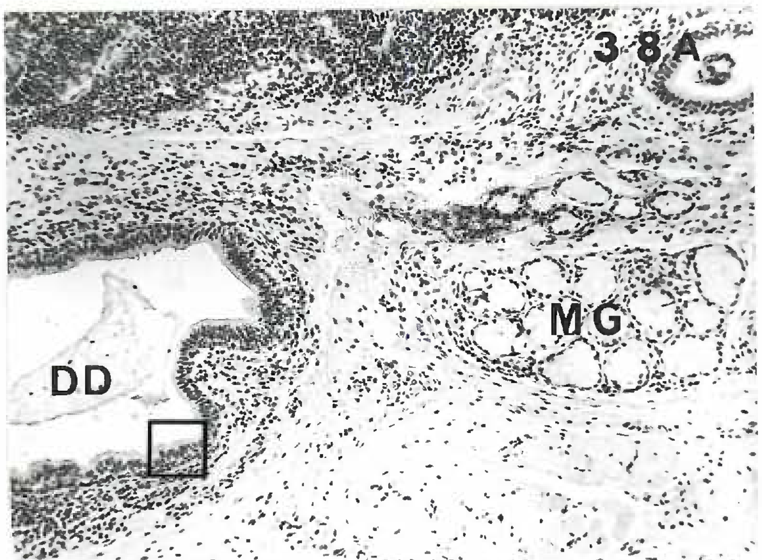
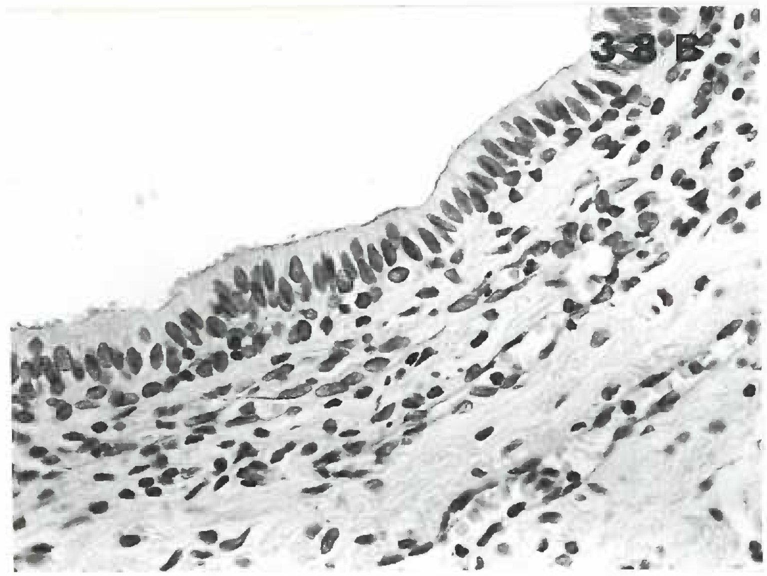
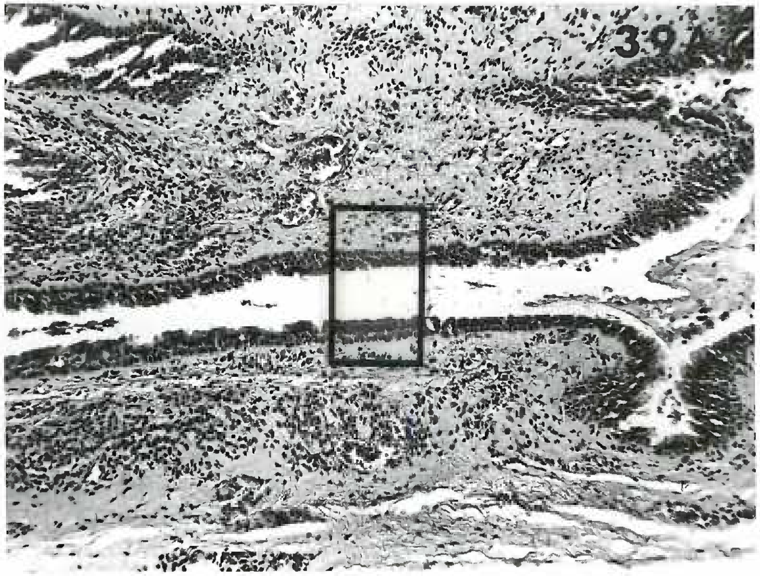

38A
MG
DD


38B

TYPES OF EPITHELIUM LINING BRANCHIAL CYSTS: INDETERMINATE AND DUCTAL

Fig. 39A. Medium power view of a portion of the lining epithelium of a branchial cyst. (Case BC204, 100X)

Fig. 39B. Higher power view of blocked area in Fig. 39A. At the bottom is a ductal type of epithelium (D) and the top is an indeterminate (I) type. (The left portion could be probable ductal and the rest, probable respiratory.) (H&E, 400X)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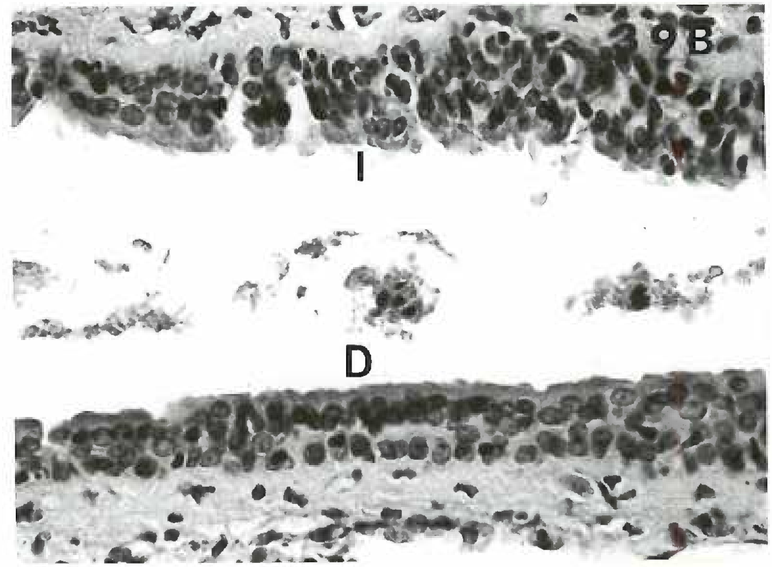

9B
I
D

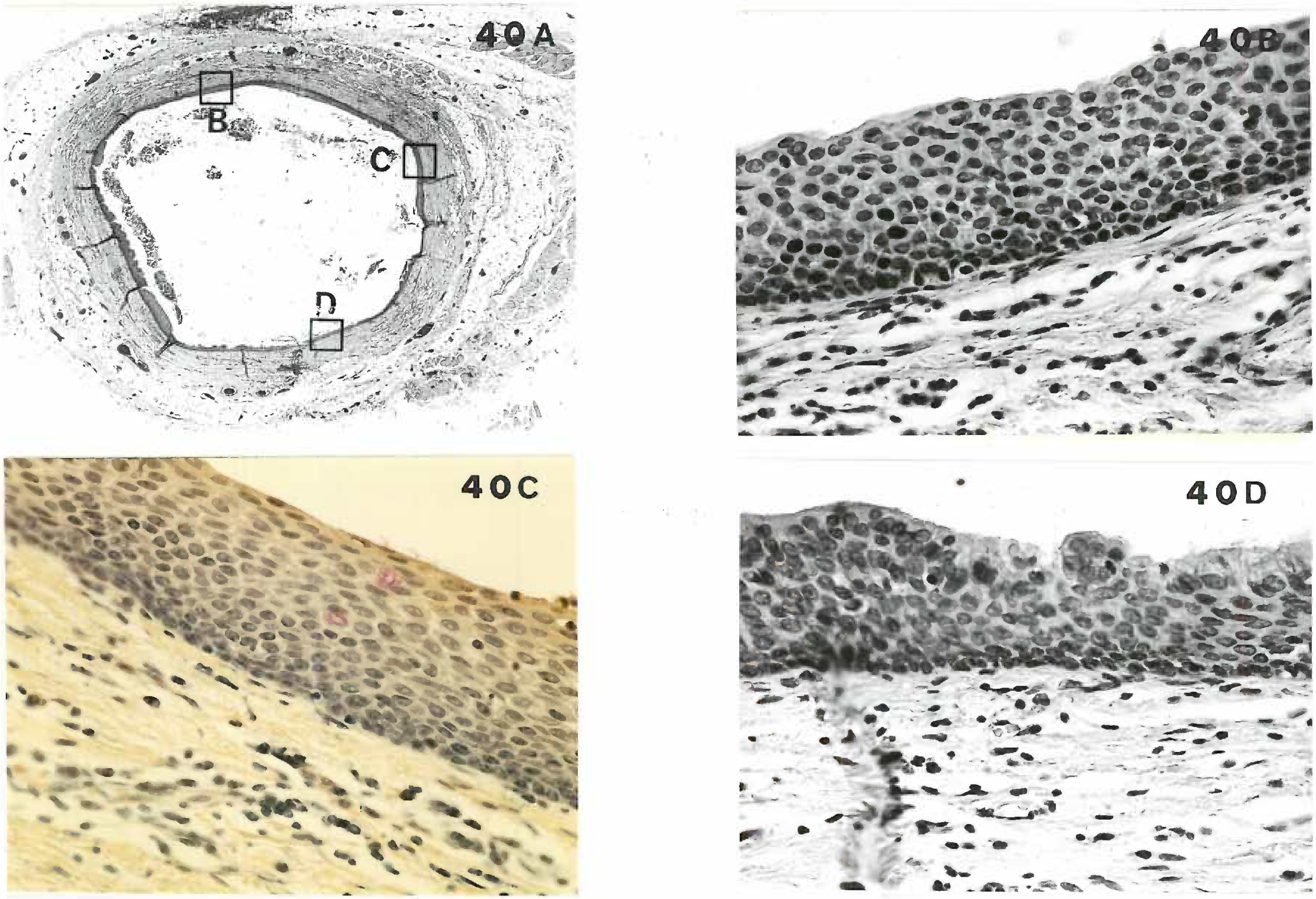
TYPES OF EPITHELIUM LINING BRANCHIAL CYSTS:
TRANSITIONAL AND RESPIRATORY

Fig. 40A. Low power view of a branchial cyst showing a lack of lymphoid tissue. (Case BC180, H&E, 12X)

Fig. 40B. Higher power view of blocked area (B) in Fig. 40A. This was classified as transitional epithelium. (H&E, 400X)

Fig. 40C. Higher power view of blocked area (C) in Fig. 40A. Note the red staining material in the squamoid epithelium. This material was interpreted as mucin. The epithelium was classified as transitional. (Mayer's mucicarmine stain, 400X)

Fig. 40D. Higher power view of blocked area (D) in Fig. 40A. Note the transitional epithelium at the left and the respiratory type at the right. The cilia becomes more dense to the right of the section. (H&E, 400X)

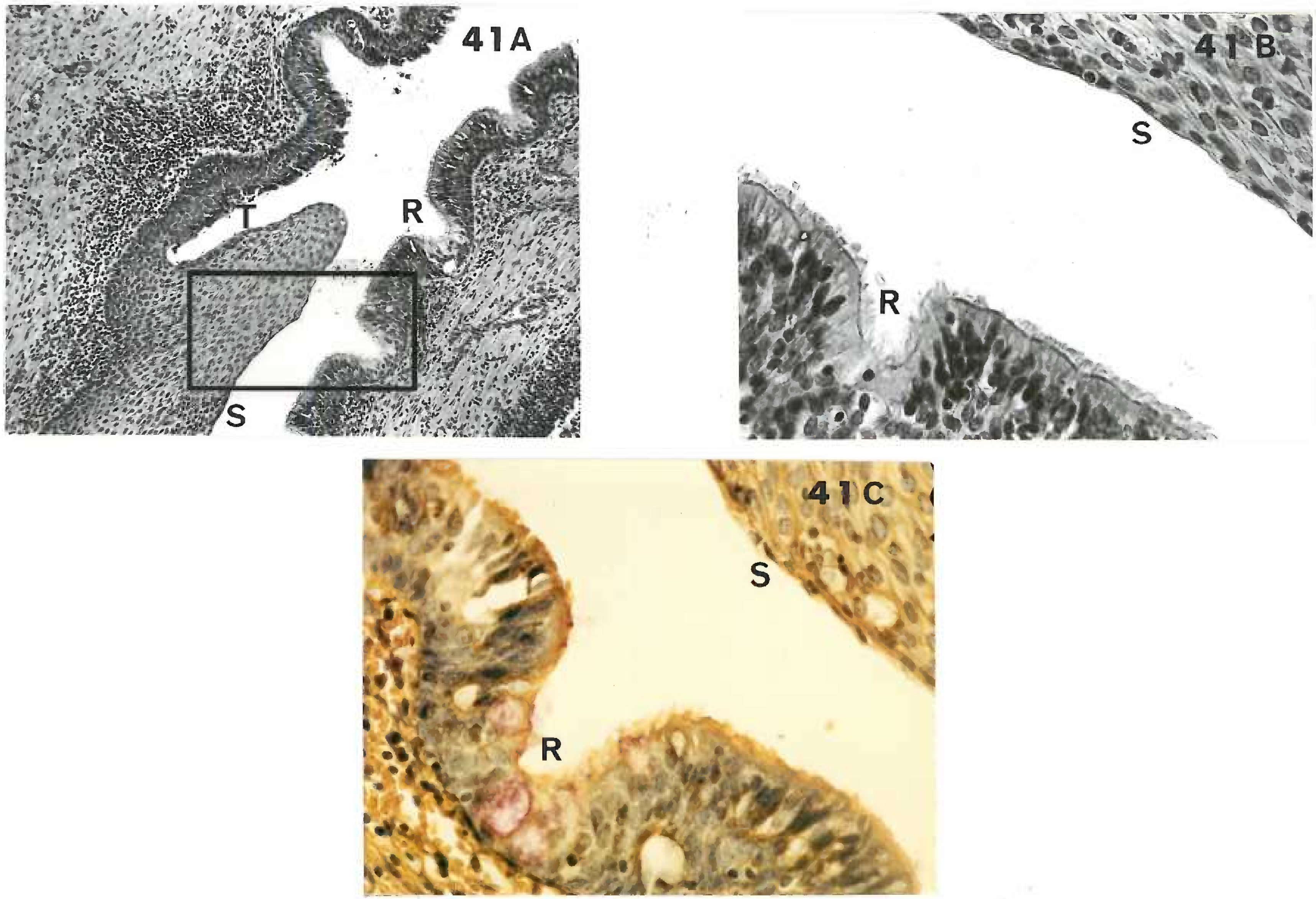

40A
B
C
D
40B
40C
40D

TYPES OF EPITHELIUM LINING BRANCHIAL CYSTS:
MODIFIED SQUAMOUS, TRANSITIONAL, RESPIRATORY

Fig. 41A. A medium power view of a branchial cyst lined by modified squamous (S), transitional (T), and respiratory (R) epithelium. (Case BC206, H&E, 100X)

Fig. 41B. Higher power view of blocked area in Fig. 41A. Note the respiratory (R) and modified squamous (S) epithelium. (H&E, 400X)

Fig. 41C. Mayer's mucicarmine stain of area shown in Fig. 41B. Note the red staining material in the respiratory epithelium. This was interpreted as mucin. The modified squamous epithelium was negative for mucin. (400X)



41A
T
R
S
41 B
S
R
41C
S
R

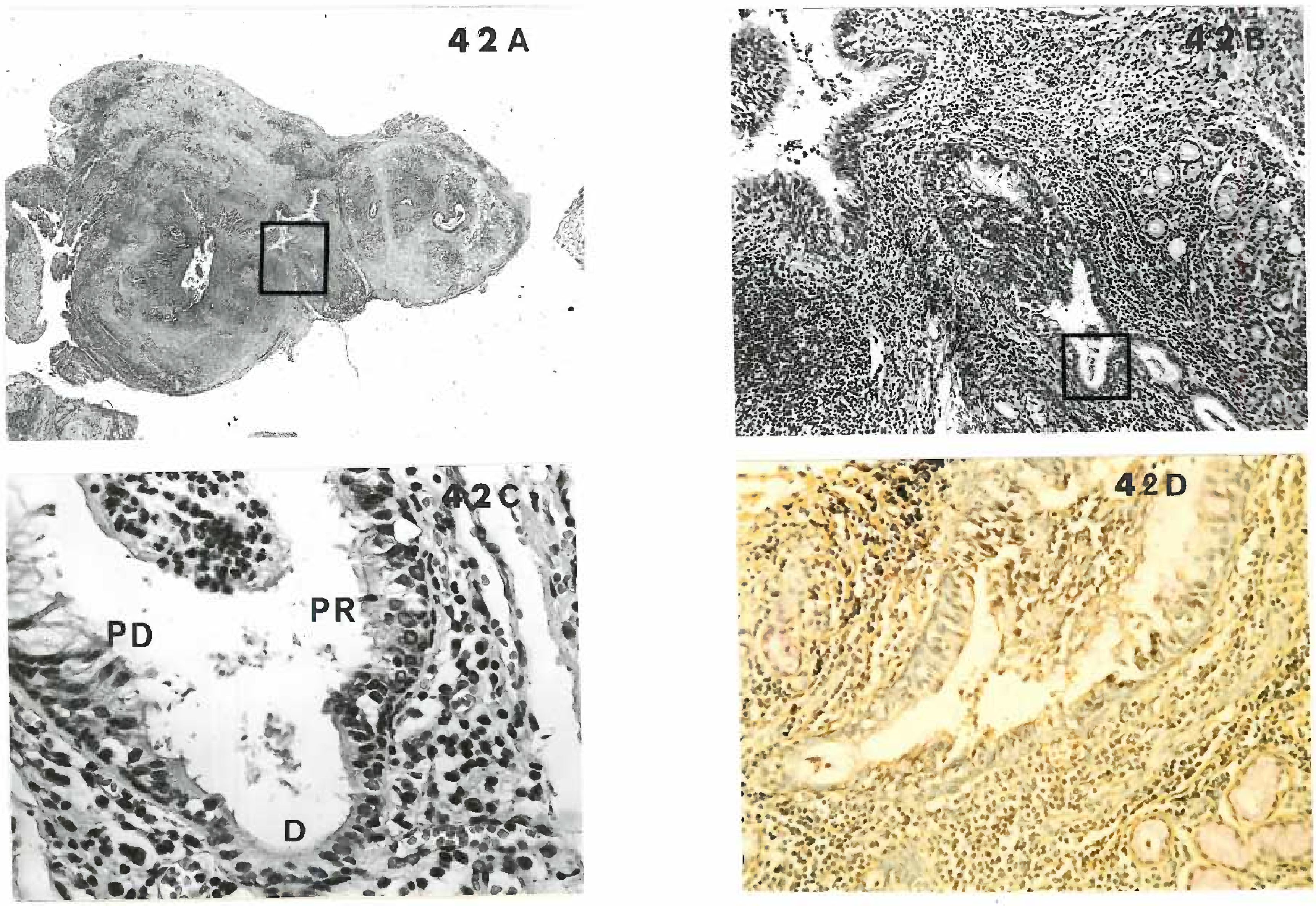
TYPES OF EPITHELIUM LINING BRANCHIAL CYSTS:
DUCTAL, PROBABLE DUCTAL, PROBABLE RESPIRATORY

Fig. 42A. Low power view of a branchial cyst. Note the mucous glands (MG). (Case BC179, H&E, 12X)

Fig. 42B. Medium power view of blocked area in Fig. 42A. The cyst in the upper left corner is lined by respiratory epithelium (R). Note the mucous glands (MG). (H&E, 100X)

Fig. 42C. High power view of blocked area in Fig. 42B. Note the ductal (D), probable ductal (PD), and probable respiratory (PR) epithelium. Also, note the structures (arrow) which could be cilia. (H&E, 400X)

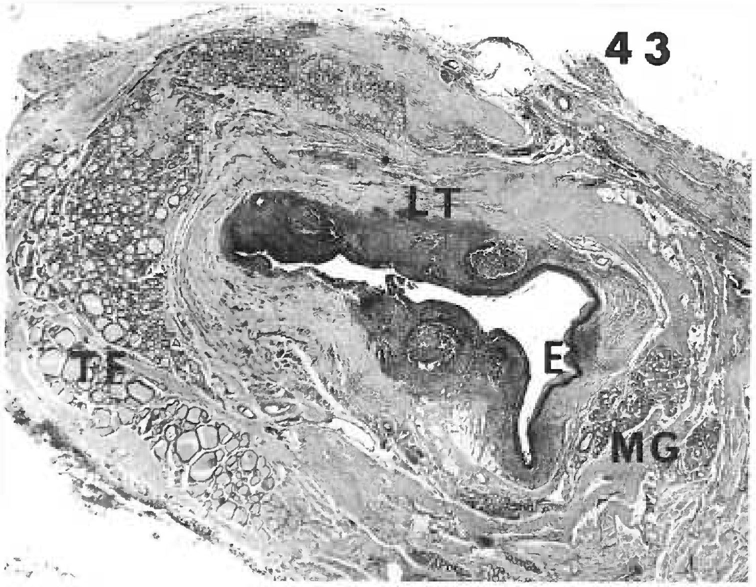
Fig. 42D. Mayer's mucicarmine stain of area shown in Fig. 42C. Note the mucicarmine positive material in and on the surface of the lining epithelium and also in the mucous glands (MG). (300X)

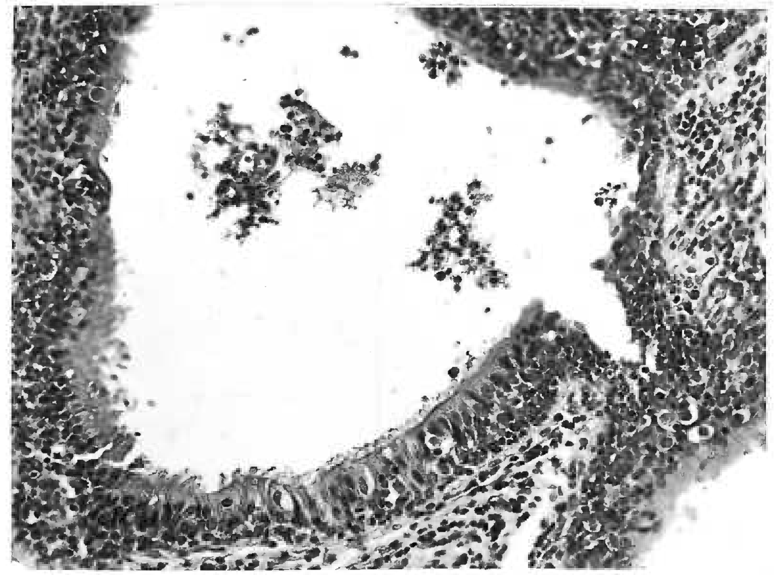

42A
42B
42C
PR
PD
D
42D

THYROGLOSSAL DUCT CYSTS

Fig. 43. A thyroglossal duct cyst showing thyroid follicles (TF) and mucous glands (MG) in its fibrous connective tissue wall. Note the association of the lining epithelium (E) and lymphoid tissue (LT) with germinal centers. (Case TD1, H&E, 12X)

Fig. 44. High power view of a thyroglossal duct cyst showing a respiratory type of lining epithelium. (Case TD2, H&E, 120X)


4 3
LT
E
MG
TE



AN ABSTRACT OF THE THESIS OF JAMES W. LITTLE for the degree of Master of Science in the department of Oral Pathology to be taken May 16, 1963.

TITLE: THE HISTOGENESIS OF THE BRANCHIAL CYST.

Approved: ______________________________
(Thesis Adviser)

The histogenesis of the branchial cyst has been a matter of controversy for many years. The most popular hypotheses concerning its origin implicate either the branchial apparatus, the thymic duct, or parotid salivary gland duct inclusions in lymph nodes. The purpose of this investigation was to accumulate data which would tend to support or reject these hypotheses with particular reference to the most recently proposed one, the salivary gland duct inclusion theory. The latter postulates that most branchial cysts take origin from parotid salivary duct tissue trapped in cervical lymph nodes during development:

Two avenues of investigation were pursued:

1) Embryos, fetuses, and lymph nodes from adults requiring radical neck dissections were studied to determine the anatomical location of cervical lymph nodes with salivary gland inclusions, and

2) 149 branchial cysts were studied microscopically using Hematoxylin-Eosin and Mayer's mucicarmine stains to identify and quantitate the types of epithelia lining their lumens.

This study indicates that the majority of branchial cysts are found in the region between the angle of the mandible and the hyoid bone just anterior to the anterior border of the sternocleidomastoid muscle. Significantly, salivary gland inclusions in this area were extremely sparse in the tissues from embryos, fetuses, and adults studied in this investigation. Conversely, parotid salivary gland inclusions in lymph nodes were plentiful in the parotid area, where branchial cysts are rarely seen.

It would seem most logical that the area with the greatest frequency of ductal inclusions in lymph nodes would contain the greatest number of cysts, if the salivary gland inclusion theory were accurate. As mentioned above, this is not the case. However, branchial cysts do arise with the greatest frequency in the area of greatest probable accumulations of epithelial rests from the branchial apparatus.

In the present series studied 105 branchial cysts were lined entirely by stratified squamous epithelium. Twenty-eight cysts were lined in part or in total by respiratory epithelium, sixteen were lined by a mixture of epithelia (excluding respiratory) or totally by an epithelium other than stratified squamous or respiratory.

Neither the results of this study nor the findings in the literature demonstrate the parotid primordia giving rise to respiratory epithelium, or more mature ductal epithelium undergoing metaplasia to respiratory epithelium. Therefore, the twenty-eight cysts which contained respiratory epithelium were not considered to have arisen from parotid gland epithelium. Conversely, the sixteen cysts which contained a mixed epithelial lining (excluding respiratory) or which were lined entirely by an epithelium other than stratified squamous or respiratory were considered to have arisen from parotid gland epithelium. These sixteen cysts could just as likely have arisen from branchial epithelium. However, to be as supportive as possible in supplying data for the statistical testing of the parotid gland inclusion theory, they were considered as parotid in origin.

The parotid inclusion hypothesis was tested on the assumption that the number of cysts of parotid origin is equal to or greater than the number of cysts of nonparotid origin. The test statistic fell into the critical region (alpha0.05, one tail test) and the hypothesis was rejected. It was, therefore, concluded that branchial cysts probably do not develop from parotid salivary gland inclusions in cervical lymph nodes.