Histology of the human nasopharyngeal mucosa*

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INTRODUCTION

Comprehensive studies on many aspects of the anatomy and embryology of the human nasopharynx are fairly well documented in the literature (Frazer, 1911*a*, *b*; Snook, 1934; Wood Jones, 1940; Oldfield, 1941; Grant, 1952; Nikhinson, 1952; Davies & Davies, 1962).

However, the microscopic anatomy of the nasopharyngeal mucosa has received little attention and some important facets of the subject are still unexplored. Despite the large number of available texts on human histology, there seems to be uncertainty regarding the type of epithelial lining of the nasopharynx. Some authors describe the nasopharyngeal lining as similar to that of the predominantly ciliated epithelium of the anterior nasal cavities (Nonidez & Windle, 1949; Carleton & Short, 1954; Arev, 1957; Davies & Davies, 1962). Other authors have shown that there are patches of stratified squamous epithelium but do not describe their extent and distribution (Maximow & Bloom, 1952; Smith, Copenhaver & Johnson, 1953; Finerty & Cowdry, 1960; Ham & Leeson, 1961). However, Maximow & Bloom (1952), Liang et al. (1962) and Yeh (1962) claim that with age the ciliated epithelium is replaced by squamous epithelium over large areas of the nasopharynx. This evidence seems inconclusive as the extent of normal distribution of the two types of epithelia is not adequately known. The issue is further complicated by the presence of a third type of epithelium in the nasopharynx, originally described by Bryant (1916) as the so-called intermediate epithelium. This epithelium was thought to be confined to a wavy ring between the oropharynx and nasopharynx. There is, therefore, a need for the study of the basic histological pattern of the nasopharyngeal mucosa in individuals of various age groups.

For this reason, and because carcinoma of the nasopharynx is especially high amongst the Chinese (Yeh & Cowdry, 1954; Yeh, 1962), it was thought desirable to present a detailed study of the histological features of the nasopharynx in the four racial groups residing in Singapore. The investigation was further designed to yield quantitative data on the distribution of the three types of epithelia in individuals of various age groups.

TERMINOLOGY

The term 'pharynx' has been applied always to include the posterior part of the nasal chambers, a region to which the term can in no proper sense be applied (Wood Jones, 1940). Epipharynx as an alternative name, although admissible to man, would not be adequate for most of the vertebrates. Negus (1961), therefore,

^{*} This investigation is part of a thesis for the Degree of Doctor of Medicine in the University of Singapore. A summary of the findings was presented at the Symposium held by the International Union Against Cancer on Nasopharyngeal Carcinoma, Singapore (3-8 August 1964).

suggested that the term posterior nasal passage should be applied instead of either nasopharynx or epipharynx.

The term *nasopharynx* in the present study is applied to that part of the posterior nasal passage which lies above the free border of the soft palate. For the purpose of this study, the limits of the posterior, anterior and lateral walls are of special importance. The following is a brief presentation of the definitions adopted by the author and the sources of information indicated.

Regions of the nasopharynx (UICC, 1963)

Anterior wall. Extends from the choanae or posterior nares to include the incomplete floor which is formed by the postero-superior surface of the soft palate.

Posterior wall (including roof). A continuous arched wall, with the roof extending from the superior margin of the choanae caudally to the level of the free border of the soft palate.

Lateral walls. There, the nasopharynx communicates with the right and the left tympanic cavities by the Eustachian tubes. The pharyngeal ostium of the auditory tube shows prominence of its posterior lip and adjacent to that is the pharyngeal recess or fossa of Rosenmüller. Passing directly downwards from the posterior lip is the salpingopharyngeal fold.

Intermediate or transitional epithelium (Bryant, 1916)

This epithelium has similar morphological features to the transitional epithelium of the urinary tract; it has five or six cell layers and no cilia. The deepest layer of cells has a cuboidal or at times a columnar shape, followed by polyhedral cells and a superficial row of rounded cells. There are no intercellular bridges and no flattening of the superficial layers.

MATERIALS AND METHODS

The findings presented in this study were based upon the histological examination of 100 autopsy specimens of the nasopharyngeal mucosa. These specimens were selected from 500 consecutive necropsies on medico-legal cases of sudden death. Cases showing any evidence of respiratory infection were excluded. Pathological changes, when present, were easily detectable in routine histological sections. In some of the specimens, however, counting of the mast cell population (Ali, 1964) was necessary to detect chronic inflammation. The sample consisted of persons whose ages ranged from 10 to 80 years. The male to female ratio was 4:1, and the four racial groups included in the present investigation were: 55 Chinese, 23 Indians, 11 Malays and 11 Europeans.

The whole of the nasopharynx and accompanying throat organs were removed en bloc by the modified method of Szanto (1944) and fixed in 10 % formol-saline solution. Identical and representative areas from each of the specimens were selected for histological examination. Mid-line sections, one each, were taken through the anterior and posterior walls, and four cross-sections of each of the left and right lateral walls. The lateral wall sections, extending from the mid-line of the posterior wall to the mid-line of the anterior wall, were taken through the pharyngeal isthmus, the pharyngeal recess and two sections along the salpingopharyngeal

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fold between the posterior nares and the ostia of the auditory tubes. Thus ten sections were taken from each specimen. The sections were stained with haematoxylin and eosin, Heidenhain's iron-haematoxylin, Van Gieson's stain, Southgate's mucicarmine, Foot's silver impregnation for reticular tissue and stains for demonstration of eleidin and keratohyalin (Carleton & Drury, 1957). Selected sections for investigation of pigment were stained by the Schmorl's reaction for melanin pigment and by the Prussian blue reaction for iron pigment (Pearse, 1960).

The stained sections were examined microscopically and the limits of the squamous and ciliated epithelia marked on the slide with indian ink. The haematoxylineosin sections were then projected on to a screen adjusted to give $\times 10$

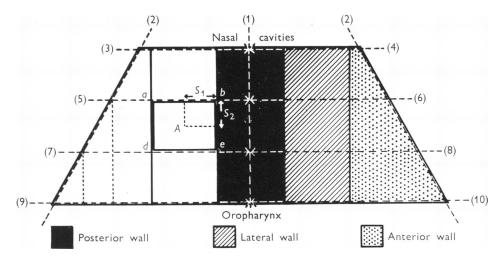


Fig. 1. Schematic line drawing of the reconstructed shape of the nasopharyngeal mucosa. The numbers (between brackets) refer to the positions of the ten histological sections taken. The three walls of the nasopharynx are shown. The total surface area of the trapezium = height (section 1) × average width ($\frac{1}{2}$ the sum of sections 3, 4, 9 and 10). The area of rectangle A is the product of any two adjacent sides, e.g. ab and be. The sum of the linear measurements of squamous patches in each of the two sides being S_1 and S_2 respectively, the product ($S_1 \times S_2$) is taken to be that part of area A which is lined by squamous epithelium.

magnification and tracings on paper were made from each of the sections. Using a planimeter, the linear measurements of the squamous epithelium, in each of the ten sections, were plotted on the tracing. The surface area of the nasopharynx was then reconstructed from the ten tracings of each specimen. The cone-shaped nasopharynx was thus invariably reconstructed into the geometrical shape of a trapezium (Fig. 1). The total surface area and the areas covered respectively by the squamous and ciliated epithelia were obtained by simple geometrical calculations. Statistical methods recommended by Hill (1956) were then used for calculating the averages and the standard deviation. Finally, the frequency of distribution was sorted out for each of the two major epithelial types in the three regions of the nasopharynx. To check the adequacy of this method, a chequered trapezoid board of a known area was taken as a model. Ten strips, corresponding to the sections taken

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from each nasopharyngeal specimen, were pasted on to a drawing board in the same order as the section tracings. Reconstruction of the surface area and the chequered pattern produced comparable results but there was an error ranging from 10 to 12 %.

RESULTS

Surface area of nasopharyngeal mucosa

The mucosa of the nasopharynx showed numerous folds and crypts as a result of which the planimeter readings were at least three times those of the apparent dimensions. The vertical measurements were only slightly altered, being consistently $5\cdot 5-6$ cm. in all age groups examined. The transverse sections, on the other hand,

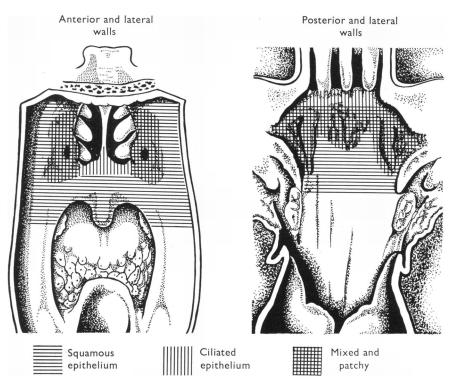


Fig. 2. Distribution of squamous and ciliated epithelia in the nasopharynx. Note the mixed and patchy pattern in the lateral and part of the posterior walls.

revealed a much larger surface ranging between 6 and 10 cm. There was no notable variation either in the apparent or in the planimeter dimensions of nasopharynges of individuals over the age of 30 years.

Calculations from the various measurements obtained in this study showed that the average area of the epithelial lining of the nasopharynx was approximately 50 cm^2 in the adult. The distribution of the squamous and ciliated epithelia showed a remarkably stereotyped pattern which is mapped in a line drawing of the three walls of the nasopharynx (Fig. 2).

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Distribution and features of the squamous epithelium

The stratified squamous epithelium was easily identifiable in the routine haematoxylin and eosin-stained sections. However, sections stained for kerato-hyalin were required in areas showing an admixture of epithelia. In all age groups examined, an average area of $60 (\pm 10) \%$ of the whole epithelial surface of the naso-pharynx was covered by stratified squamous epithelium (Fig. 3). The distribution

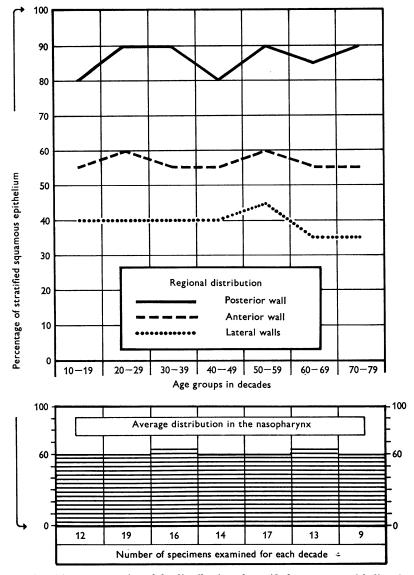
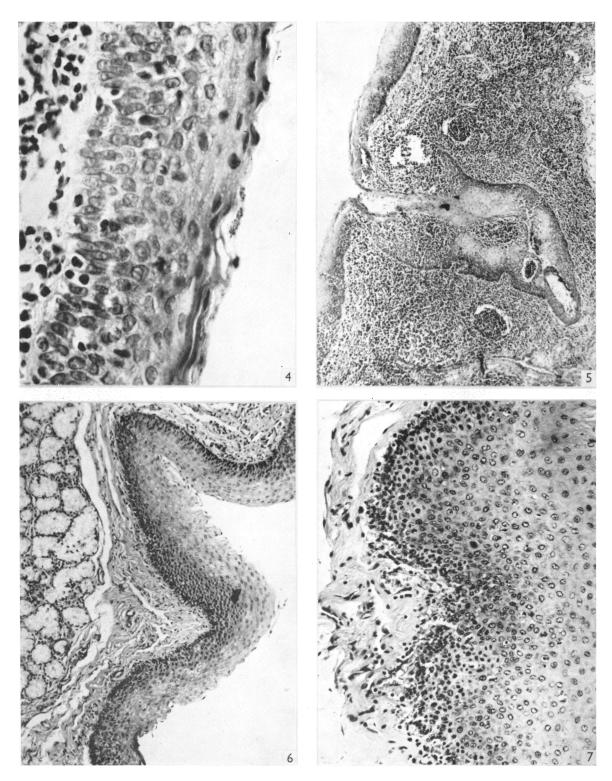


Fig. 3. Graphic representation of the distribution of stratified squamous epithelium in the three regions of the nasopharynx correlated with age. The histogram at the bottom shows the average distribution and the number of specimens examined in each decade.



of squamous epithelium did not seem to be affected appreciably by the factors of sex and race. Except for the part adjacent to the posterior nares, almost 60 % of the anterior wall of the nasopharynx was lined by stratified squamous epithelium. In the posterior wall, 80-90 % of the surface area was lined by squamous epithelium. The lining of the pharyngeal tonsil and that of both lateral walls showed a remarkable pattern of alternating patches of ciliated and squamous epithelia which were often separated by islets of intermediate epithelium (Fig. 3).

The squamous epithelium was largely non-keratinizing except in the pharyngeal crypts (Figs. 4, 5). However, areas of keratinization and acanthosis of the epithelium were present in the nasopharyngeal mucosa of individuals over the age of 50 years (Figs. 6, 7). These keratinized patches were in the posterior and lateral walls and also within the pharyngeal crypts. The anterior wall was almost entirely devoid of keratinization and other epithelial changes.

In all specimens, patches of the epithelial lining of the pharyngeal and tubal tonsils showed migration of lymphocytes between the epithelial cells (Figs. 8, 9). In those areas, a break in the reticular fibres of the basement membrane was demonstrable by the use of silver impregnation stain (Figs. 10, 11).

Well-formed blood vessels were found within patches of the squamous epithelium in the posterior and lateral walls. These vascular channels were situated close to the surface between the stratified layers of the epithelium. Although often seen in the form of vascular papillae (Fig. 12) in many areas they showed no apparent connexion to the vascular bed in the underlying tunica propria.

Distribution of ciliated and transitional epithelia

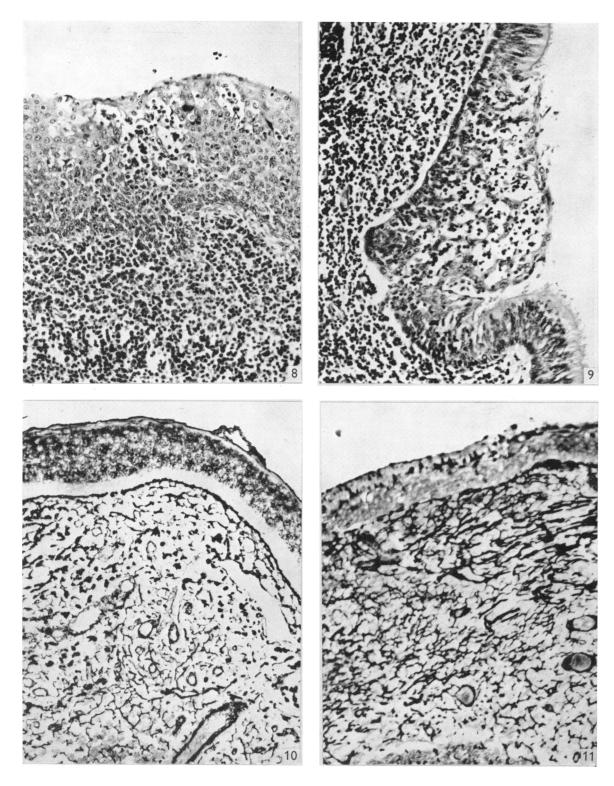
The pseudo-stratified ciliated columnar epithelium, stained with mucicarmine, had an appearance similar to that of the upper respiratory epithelium and there were numerous goblet cells between the ciliated cells (Fig. 13). Here again, the character and distribution of the ciliated and transitional epithelia were unaffected by factors of sex and race. Generally, the ciliated epithelium had a thickness of $100-120\,\mu$ m. In the lateral wall, overlying the pharyngeal recess, the goblet cells were markedly increased in number and the undulating epithelium covering it averaged about $80\,\mu$ m in thickness. At least a third of the anterior wall, abutting on to the nasal choanae, and a narrow strip of the roof were exclusively lined by ciliated epithelium. Approximately $40\,\%$ of the anterior wall and only $15-20\,\%$ of the posterior wall were covered by ciliated epithelium. Almost half the area of the lateral walls was lined by ciliated epithelium distributed in irregular patches alternating with islets of squamous and transitional epithelia (Figs. 14-16).

Fig. 4. Photomicrograph of a section from the mucous membrane of the nasopharynx showing an area of non-keratinizing stratified squamous epithelium with distinct flattening of squamous cells on its free border. Haematoxylin and cosin. \times 500.

Fig. 5. Section through the pharyngeal tonsil showing an epithelial *crypt* lined by keratinized stratified squamous epithelium. The surface epithelium of the nasopharynx is shown to the left. Haematoxylin and eosin. $\times 45$.

Fig. 6. An area of keratinizing epithelium in the posterior wall of the nasopharynx in a Chinese male aged 70 years. Haematoxylin and cosin. $\times 75$.

Fig. 7. Increased cellularity and acanthosis of the nasopharyngeal squamous epithelium taken from the same case as in Fig. 6. Haematoxylin and eosin. $\times 150$.



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The transitional or intermediate epithelium had some features similar to those of the transitional epithelium of the urinary system (Fig. 17). The stratified nature of this epithelium was shown clearly in contrast to that of the pseudo-stratified ciliated variety. However, its nuclei showed vertical alignment similar to that of the ciliated epithelium but were easily differentiated, even under the low power, from the flattened cells of the squamous epithelium. There were also no cilia and no intercellular bridges. Although histologically separable from the squamous epithelium, the cells of the intermediate epithelium contained keratohyalin and occasionally keratin. An aggregate of intermediate epithelium formed a wavy ring separating the nasopharynx from the oropharynx. The lateral walls also contained numerous islets of the intermediate epithelium. Similar distribution of the intermediate epithelium was seen in the mucous lining of the pharyngeal tonsil in the posterior wall.

Pigmentation in the mucosa

The basal layer of the epithelium showed dark brown pigment in seventeen of the hundred specimens examined. The pigment was found in patches of the squamous, ciliated and intermediate epithelia. The pigmented areas of the epithelium were confined to the posterior and lateral walls. Although most of the pigment granules were condensed in the basal layer, the ciliated and intermediate epithelia showed some pigment even amongst the upper layers of cells. All the pigmentcontaining specimens gave positive results for melanin stain by the Schmorl's reaction while the Prussian blue reaction for iron was negative. Only three specimens from individuals of Chinese origin showed pigmentation while fourteen were Indians and Malays (that is 41% of the specimens from Indians and Malays). None of the Europeans showed pigmented epithelium.

The tunica propria

The tunica propria of the nasopharynx had a framework of collagenous and elastic fibres and numerous blood and lymphatic vessels. By the use of a modified Dominici stain (Ali, 1964), large numbers of mast cells were found throughout the tunica propria. Mixed mucous and serous secreting glands were found in the submucosal space and occasionally deeper to the muscle bundles.

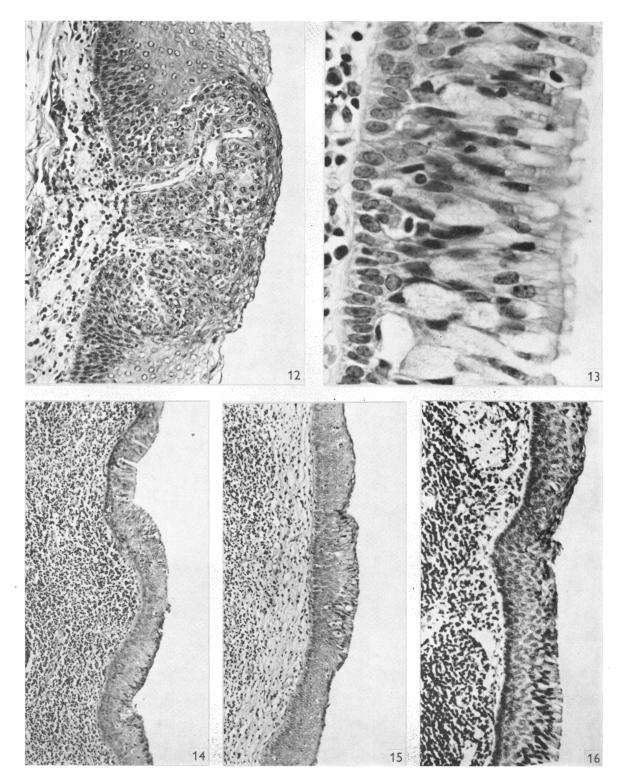
Three pharyngeal tonsils were formed by the accumulation of lymphoid tissue in the median dorsal wall and within, or close to, the orifices of the Eustachian tubes. Other parts of the tunica propria showed collections of lymphocytes without the

Fig. 8. A patch of the nasopharyngeal squamous epithelium showing lymphocytic migration from the underlying lymphoid follicles. Haematoxylin and eosin. \times 150.

Fig. 9. Migration of lymphocytes in an area of the nasopharynx which is lined by pseudo-stratified ciliated epithelium. The ciliated cells are widely separated by lymphocytes, causing the epithelium to bulge outwards. Haematoxylin and cosin. $\times 150$.

Fig. 10. Reticular fibres in the basement membrane of the normal nasopharyngeal mucosa. Note the sharp condensation of reticular fibres in the form of a continuous cord beneath the epithelium. Foot's silver impregnation. $\times 150$.

Fig. 11. Section from an area of lymphocytic migration showing fragmentation of the reticular fibres of the basement membrane. Foot's silver impregnation. $\times 150$.



formation of follicles. The epithelial lining of the pharyngeal tonsils showed numerous folds separating the lymphoid follicles and there were also deep crypts similar to those of the palatine tonsils. Ducts of the mucous glands usually open into the mucosal folds while the crypts formed culs-de-sac epithelial ingrowths lined by keratinizing squamous epithelium.



Fig. 17. High-power view of the intermediate or transitional epithelium. The scalloped appearance of the surface layer of cells is clearly shown. Note the vertical alignment of the cells and the absence of cilia. Haematoxylin and eosin. \times 500.

All the specimens showed the presence of lymphoid masses in the nasopharynx even in individuals of 80 years of age. The persistence of pharyngeal tonsils was accompanied by deepening and branching of the crypt system.

Fig. 12. Non-keratinizing epithelium of the nasopharynx showing vascular papillae reaching close to the surface of the epithelium. Haematoxylin and cosin. \times 150.

Fig. 13. High-power photomicrograph of the pseudo-stratified columnar ciliated epithelium of the nasopharynx. Note the distinct cilia, basement membrane and numerous goblet cells. Southgate's mucicarmine. \times 500.

Fig. 14. Patches of ciliated and squamous epithelia separated by transitional epithelium. Ciliated epithelium is shown above and squamous below. Haematoxylin and eosin. \times 75.

Fig. 15. A patch of mixed (ciliated and transitional) epithelia in an area of the naso-pharynx lined by squamous epithelium. Haematoxylin and eosin. \times 75.

Fig. 16. Photomicrograph of a section showing the junction between squamous epithelium (above) and ciliated epithelium (below). Haematoxylin and eosin. $\times 150$.

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DISCUSSION

The technique developed for this investigation was planned to yield quantitative data, and permit, by means of statistical methods, conclusions to be drawn. The object was to calculate the total area of the nasopharyngeal mucosa and also find the frequency distribution of the two major types of epithelia. The nature, size and selection of the sample was considered first. According to Hill (1956), the larger the random sample, the more accurately are we likely to reproduce the characteristics of the universe from which it is drawn. Thus a sample consisting of 100 nasopharyngeal specimens was selected from four races: this was also representative of the various age groups. Calculation of the total area of the nasopharyngeal mucosa posed no particular problem. Only three measurements were required from each specimen for the geometrical calculation of the trapezium-shaped nasopharynx. Each of the measurements was obtained from the relevant histological section and the mucosal folds 'ironed out' by the use of a planimeter. An arithmetic mean was then calculated for each decade and also from the sum of the 100 observations. To determine the frequency distribution of the two types of epithelia, it was necessary to increase the number of samples and thereby minimize the error. Sections were thus taken in four planes from each of the three anatomical regions of the nasopharynx. Thus, twelve samples were taken from each nasopharyngeal specimen. The area of each sample, roughly a rectangle, was calculated from the measurements of any two adjacent sides (Fig. 1). The product of the linear measurements of squamous patches in the two sides was taken to represent the proportion of the squamous epithelium. The values calculated from these samples were not absolute and were subject to the laws of chance. The standard deviation was then calculated by the method for grouped series (Hill, 1956). A paper model was also used to check the validity of the method. Because of an error of approximately 10 %, inherent to this method, there was a wide scatter from the arithmetic mean.

Area of the nasopharyngeal mucosa

The planimeter measurements of the vertical and transverse dimensions of the nasopharyngeal cavity were about three times those given by Nikhinson (1952) and Adams (1958). Consequently, the actual area of the nasopharyngeal mucosa was also larger than the apparent surface area. Minear, Arey & Milton (1937) reported that the surface area of the entire pharynx was approximately 45 cm². However, the average calculations in this study showed that the actual surface area of the nasopharyngeal mucosa alone was 50 cm² in the adult.

The transitional epithelium

The present study revealed that the transitional epithelium was almost always present between the squamous and ciliated epithelia. Because of the patchy distribution of the squamous and ciliated epithelia in the lateral walls of the nasopharynx, numerous islets of transitional epithelium were found all over the salpingopharyngeal fold and the pharyngeal recess. A ring of transitional epithelium, similar to that described by Bryant (1916), was also present in the boundary line between the nasopharynx and the oropharynx. Keratohyalin, and occasionally keratin, were present in the superficial layer of the transitional epithelium. There was evidence, therefore, that the transitional epithelium is histochemically related to the squamous epithelium. According to Rothman (1954), whenever a non-cornifying epithelium is transformed into squamous epithelium, keratohyalin and eleidin appear in the transitional layer as soon as the epithelium acquires cornifying potentiality. However, it was not possible on the available evidence to postulate that the transitional epithelium might eventually become squamous.

The stratified squamous epithelium

There was no variation in the distribution of squamous epithelium in the nasopharynx of normal individuals whose ages ranged between 10 and 80 years. The findings in this study showed that 60 (\pm 10) % of the total mucosal surface is lined by squamous epithelium. In stillborn and newborn infants, however, Yeh (1962) showed that the nasopharynx is covered solely by pseudo-stratified columnar epithelium. Subsequently, Yeh (1964) found squamous epithelium in histological sections taken from the nasopharyngeal mucosa of several newborn infants. Although patches of squamous epithelium might be found in the nasopharyngeal mucosa of infants, most of the change would be completed by the end of the first decade of life. An optimal level is thus maintained throughout life unless further increased by pathological metaplasia. According to Smith et al. (1953) and Arey (1957), normal squamous transformation in the nasopharynx has been attributed to the circumstance that the soft palate frequently comes in contact with the posterior pharyngeal wall. Moreover, based on the findings of Lion (1950), the nasopharyngeal arch is usually low in infants and that enables the pharyngeal tonsillar masses to touch each other for months or years. Nikhinson (1952) has also shown that the maximum growth of the nasopharynx is in height and width but not in depth. Therefore, mechanical contact between mucous surfaces in the nasopharynx is maximal during infancy and childhood.

In persons over the age of 50 years, epithelial acanthosis and keratosis were frequently encountered in many parts of the nasopharynx. But, in younger age groups, keratinization of the squamous epithelium was only seen in the nasopharyngeal crypts. Although keratinization has often been found in association with pathological lesions, its mere presence does not signify disease or tendency toward neoplasia. According to Johnson (1961), keratinization is a form of cytodifferentiation and must no longer be regarded simply as a degenerative change. Acanthosis of the squamous epithelium has been related to the stripping of the keratinized layer of the squamous epithelium. Experimental evidence reported by Pinkus (1952) confirmed that the loss of keratinized cells was a primary stimulus to epidermal proliferation.

Blood vessels between the cells of the squamous epithelium were frequently observed in sections of the posterior and lateral walls of the nasopharynx. Although these were similar to the so-called intraepithelial blood vessels illustrated by Panico (1931), there was no conclusive evidence that such vascular channels were in fact intraepithelial. Since vascular papillae were often seen beneath thinned patches of the mucosa, sections taken through areas of mucosal folding might give the impression that the blood vessels were intraepithelial.

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Melanin pigmentation

The presence of melanin pigment in the basal cells of the squamous, ciliated and transitional epithelia of the nasopharynx was observed in 17% of the specimens examined. Specimens collected from Indians and Malays accounted for 14% of the pigmented nasopharyngeal mucosae while the remaining 3% were from Chinese. However, only 41% of the specimens from Indians and Malays revealed mucosal pigmentation. Melanin, normally present in the skin, has been found in the mucosa of the anterior nasal cavities (Friedmann, 1961), but there are no reports to show its presence in the nasopharynx.

Nasopharyngeal tonsils and crypts

The development, hypertrophy and involution of Waldeyer's ring received the attention of many workers (Snook, 1934; Mathies, 1935; Hollender & Szanto, 1945; Semenov, 1953; Hollender, 1959). Mathies (1935) reported that the nasopharyngeal tonsils develop later than the other members of Waldeyer's ring and undergo involution after puberty. According to Hollender & Szanto (1945), Nikhinson (1952) and the observations in the present study, nasopharyngeal tonsils were often found in persons past 70 years of age. However, hypertrophy of the nasopharyngeal lymphoid tissue in the adult may not always be associated with clinical symptoms. Anatomical data presented by Lion (1950), Nikhinson (1952) and Adams (1958) have shown that the nasopharyngeal cavity in the adult becomes sufficiently large to accommodate the hypertrophied tonsillar masses with only a remote risk of obstruction.

The presence of crypts in the pharyngeal tonsils has not been fully accepted by many histologists. Killian (1888) stated that there were superficial furrows in the nasopharyngeal mucosa but these were different from the epithelial ingrowths of the palatine tonsils. Schaeffer (1928) reported that the deep crypts of the nasopharynx were usually connected with the openings of the excretory ducts of the mucous glands. Arey (1947) described culs-de-sac folds in the mucosa of the nasopharynx and named them 'cryptanalogues'. All the specimens examined in the present study revealed a system of true crypts such as are seen in the palatine tonsils. Further details on the structure and clinical significance of the nasopharyngeal crypts were published elsewhere (Ali, 1965). The existence of the nasopharyngeal crypts cannot be over-emphasized since two widely read and recent text-books of histology (Maximow & Bloom, 1952; Ham & Leeson, 1961) still maintain that there are no crypts in the nasopharyngeal tonsils.

SUMMARY

The results of this study were based on the histological examination of nasopharyngeal mucous membranes obtained from 100 medico-legal necropsies on persons between the ages of 10 and 80, belonging to four racial groups. A technique has been developed to yield quantitative data on the distribution of the various types of epithelia in the nasopharynx.

In all age groups examined, irrespective of sex and race, 60 % of the total

epithelial surface of the nasopharyngeal mucosa was lined by stratified squamous epithelium. Alternating patches of squamous and ciliated epithelia separated by islets of transitional epithelium were seen in the lateral walls and the roof of the nasopharynx. Transitional epithelium was also present in a narrow zone between the oropharynx and the nasopharynx.

The squamous epithelium of the nasopharyngeal mucosa was largely nonkeratinizing except in deep crypts. However, keratinization and acanthosis of the squamous epithelium was seen in individuals over the age of 50 years.

Three tonsillar masses in the median dorsal wall and both lateral walls of the nasopharynx were present in all the age groups examined. Each of the tonsillar masses had a system of crypts similar to that of the palatine tonsils.

Melanin pigmentation has been demonstrated in all three types of epithelia in 17 % of the specimens. The majority of the specimens (14 \%) were from Indians and Malays and only 3% were from Chinese.

The author is indebted to Professor K. Shanmugaratnam, Head of the Department of Pathology, for his interest and advice, and to Professor R. Kanagasuntheram, Head of the Department of Anatomy, for revising the manuscript. Thanks are due to Doctors C. S. Muir and M. Ramsbotham for the German and Russian translations. The technical assistance of Mrs Mary Low, Mr V. Nalpon, Mr T. C. Tan, Mr K. S. Chew and Mr K. G. Lim is acknowledged. Mr P. A. Samuel typed the manuscript.

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