Morphological Assessment of Pathological Changes within the Rat Larynx*1

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ABSTRACT

Routine histological examination of the rat larynx, from inhalation toxicity and carcinogenicity studies, has been routinely performed in this laboratory for over 15 yr. This work has identified the larynx as an important target organ with a number of pharmaceuticals, industrial chemicals, agricultural chemicals, and environmental pollutants. The rat larynx contains 5 epithelial types: stratified squamous; low squamoid; respiratory; and 2 forms of pseudostratified cuboidal epithelium. Each epithelial type has a specific location within the relatively complex anatomical configuration of the larynx. The detection of induced changes requires a consistent, thorough and detailed histological examination in order to identify the often subtle changes in the distribution of these epithelial types. Induced lesions show distinct predilection sites, including the ventro-lateral regions anterior to the ventral pouch and the inner aspects of the arytenoid projections. Lesions commonly involve degeneration of the original epithelial cells with subsequent regeneration; hyperplasia and squamous metaplasia. In more severe reactions, the larynx may illustrate epithelial ulceration with exudation. To some extent the changes commonly observed are dependent upon the duration of the study and the dose level employed, rather than the individual compound. Recovery or regression of induced changes is variable and dependent upon the time scale involved and severity or type of initial lesion.

Keywords. Metaplasia; ulcerative laryngitis; inhalation toxicity; toxicological pathology

ANATOMY

The development of the rat larynx has been described (15). The relatively complex anatomical configuration of the airway reflects the shape and arrangement of its cartilaginous framework (7).

Craniially, the laryngeal vestibule is round in shape and bordered ventrally by the elastic epiglottis, laterally by the ary-epiglottic folds, and dorsally by the cranial end of the arytenoid cartilages. The epiglottis is triangular in shape with a straight base and long curved apex, and extends from its basal articulation with the inner surface of the thyroid cartilage cranially to the palate. A pair of lateral wings from the epiglottis pass along the ary-epiglottic folds, which in turn join the dorsal aspects of the arytenoid cartilages.

The bracelet-shaped thyroid cartilage encloses the other laryngeal cartilages laterally and ventrally. Its lateral aspect is elongated at both ends to form superior and inferior horns. A pair of short projections arise from the ventral aspect of the thyroid cartilage close to its articulation with the epiglottis and pass laterally almost parallel with the ventral mucosa. The characteristic shape of the airway at this level is determined by the position of the arytenoid cartilages which project prominently into the lumen.

At the level of the arytenoid cartilages, at the base of the epiglottis, a pouch-like extension of the laryngeal lumen is found in the ventral wall. The entrance of this pouch is maintained by a free, "U-shaped" cartilage. The processes of the "V-shaped" arytenoid cartilages articulate with the cranial surface of the dorsal cricoid cartilage. Two additional pairs of processes project from the arytenoid cartilages: the corniculate, cranially; and the vocal, caudally. The vocal folds project ventrally from the vocal processes of the arytenoid cartilages towards the midline of the thyroid cartilage (1, 15). The laryngeal lumen at this level is diamond shaped.

Below the vocal folds, the rounded shape of the lower laryngeal lumen is maintained by the cricoid cartilage which articulates with the inferior horn of the thyroid cartilage.

HISTOLOGY

By light microscopy, 5 types of epithelium are identifiable (12). The distribution of these types is...
relatively complex. Squamous epithelium is found over the majority of the epiglottis, over the arytenoid projections, in the lateral ventricles, in the dorsal region, and in a narrow band over the "U-shaped" cartilage cranial to the ventral pouch. The degree of stratification varies, being most pronounced in the dorsal region and in the lateral ventricles. The surface of the superficial squamous cells is covered by microridges or microplicae (3, 12). These ridges are poorly developed in areas of apparent recent desquamation. This suggests their development may be related to cell maturation. Globule leucocytes located in the basal region are less frequently seen in squamous epithelium than in other epithelial types. Taste buds are present in areas of squamous epithelium, particularly the epiglottis (14).

The epithelium over the vocal folds is of a low "squamoid" type with 2 distinct cell layers—darker basal cells and more lightly stained surface cells. The term squamoid is used as it does not conform to the characteristics of squamous epithelium.

Respiratory ciliated epithelium occupies the majority of the larynx caudal to the vocal folds and is also found as small isolated islands in the ventrolateral aspects at the base of the epiglottis. This corresponds to the point of entry of submucosal gland ducts (12). The epithelium contains ciliated, intermediate, serous and basal cells, with a few brush cells and Kulchitsky cells identifiable with the electron microscope (12). Goblet cells are rare. Globule leucocytes are frequently seen in respiratory epithelium.

A type of cuboidal epithelium forms intermediate or transition zones between areas of squamous and respiratory epithelium. The most extensive area of this epithelial type is found in the ventrolateral aspects opposite the arytenoid projections. The polygonal, non-ciliated cells have distinct cell margins and a slightly convex apical surface with short microvilli. This epithelium is of variable height sometimes only 2 cell layers thick, but may also have a pseudostratified appearance. Generally, the height decreases caudally towards the ventral pouch. Variable numbers of globule leucocytes are present.

A second type of cuboidal epithelium lines the ventral pouch. This epithelium has a distinct darker basal layer and a lighter superficial layer. The epithelium is usually folded. Variable numbers of globule leucocytes are present.

Thus, generally squamous epithelium is found in areas likely to experience "wear and tear" and respiratory epithelium in areas likely to be subjected to little abrasion or insult. Cuboidal epithelia represent epithelial types intermediate between these 2, both in terms of location and resistance to injury. The major exception to this pattern i.e., respiratory epithelium in the ventrolateral region at the base of the epiglottis, corresponds to one of the major sites of induced change (see later).

Submucosal glands, composed of mucous and serous cells are extensive in the caudal larynx, whereas in the epiglottis they are predominantly composed of mucous cells (2).

Mitotic Indices

Using colchicine, distinct differences between the different epithelial types in terms of their mitotic activity are demonstrable (10), not surprisingly, stratified squamous epithelium has the highest rate followed by the squamoid epithelium of the vocal folds; the cuboidal epithelium in the ventrolateral region; the cuboidal epithelium in the ventral pouch; and finally, by the respiratory epithelium.

Histological Examination

In this laboratory, a technique has been used over several years which facilitates the thorough and consistent examination of the larynx, to include the major predilection sites for induced injury. Briefly, the cranial portion of the epiglottis is removed and the larynx embedded transversely, cut surface downwards. During sectioning, the block is trimmed and initially 5 step-sections taken at irregular intervals to include the base of the epiglottis. Secondly, the position of the arytenoid projections is used to take a further 5 step-section. The arytenoid projections in fact "point" at one of the most susceptible areas—the ventrolateral aspects. The block is again trimmed and another 5 step-sections taken to include the vocal folds and the caudal larynx, using the shape of the laryngeal lumen as a guide.

Spontaneous Pathology

Spontaneous i.e., non-treatment induced changes, are relatively rare in the larynx of SPF barrier maintained rats (11). A few rats have small aggregates of inflammatory cells in the ventrolateral regions at the base of the epiglottis. In some pronounced cases, these aggregates may give rise to a lymphoepithelioma.

Passively inhaled fur (hair) appears to have a predilection to accumulate in the ventral pouch where a granulomatous reaction is induced.

Some workers have reported up to a 10% incidence of squamous metaplasia in control rats (5). However, in this laboratory a figure of less than 1% is considered more typical, even in 2-yr-old animals.

Toxicological Pathology

The larynx is a major site of induced changes in rats exposed by inhalation to pharmaceuticals, industrial chemicals, and agrochemicals (6). It should
FIG. 1.—Degenerate cells of the original ciliated epithelium loosely attached to the underlying squamous metaplastic epithelium. H&E. × 150.

perhaps be emphasized that few compound specific lesions are induced. The laryngeal mucosa generally only reacts to insult by the development of a limited range of lesions. Often the dose level is a far more important factor than the nature of the compound in the development of lesions.

A thorough knowledge of the normal laryngeal anatomy and histology, and a consistent detailed histological sampling, are essential for the detection of subtle changes in epithelial type distribution. Histologically, an induced squamous metaplastic epithelium may be completely indistinguishable from a normal squamous epithelium at an adjacent site. The distribution of induced lesions shows distinct predilection sites and is related to the anatomical, histological, and airflow characteristics of the larynx (9). These sites include the inner aspects of the arytenoid projections, which are lined by squamous epithelium, the ventrolateral regions at the base of the epiglottis covered by ciliated epithelium and areas anterior and lateral to the ventral pouch, which are covered by cuboidal epithelium.

At the latter 2 sites, the most commonly induced laryngeal lesions involve degeneration of the original epithelial (cuboidal and ciliated) cells, with subsequent hyperplasia and squamous metaplasia. This metaplastic epithelium may show superficial keratinization. The metaplastic transformation takes place surprisingly quickly, in some cases within 3 days of commencement of exposure. During the development of this lesion, a mild infiltration of neu-
trophils sometimes occurs, and globule leucocytes disappear from the epithelium. The proliferative response is so rapid that the damaged cells of the original epithelium are “lifted” from the basement membrane and they may be seen loosely attached to the newly formed underlying metaplastic epithelium (Fig. 1). Once formed, the squamous metaplastic epithelium may assume a folded appearance with the enmeshing of capillaries within the folds.

In areas of squamous epithelium, induced changes generally involve an increased epithelial height, sometimes with a more pronounced stratification and superficial keratinization. This is commonly seen along the inner aspects of the arytenoid projections and in the narrow band of squamous epithelium in the mid-line of the ventral region, cranial to the ventral pouch.

Assessment of mitotic activity using colchicine provides dramatic confirmation of the rapidity with which proliferative lesions develop following injury. A significant increase in mitotic activity is detectable, in susceptible areas, 24-hr post-exposure with a peak activity at 3-days post-exposure. From this time, the mitotic activity decreases rapidly and finally becomes stable.

In more severe reactions, the larynx illustrates epithelial ulceration and necrosis of the underlying tissue. This is accompanied by a pronounced inflammatory exudate (Fig. 2) which in some cases may obliterate the laryngeal lumen (6). This type of change is produced by lithium combustion aerosols (13). With ulcerative lesions secondary bacterial colonization may occur (13). Changes may also be seen in the laryngeal cartilages which may undergo necrosis along with the overlying mucosa. The “U-shaped” cartilage at the entrance to the ventral pouch is particularly prone to this change, presumably due to its superficial location immediately beneath the epithelial surface. The necrotic appearance of the cartilage may persist even after repair of the overlying mucosa. Necrotic arytenoid cartilages may herniate through the overlying necrotic mucosa into the laryngeal lumen (Fig. 3).

Repair following ulceration is characterised by the development of a low epithelium composed of a single layer of flattened cells continuous with the epithelium at the margins of the lesion. Granulation tissue is present in the lamina propria. At sites where submucosal gland ducts are present, the reparative epithelium is usually continuous with the cells lining the ducts. Subsequent continued repair results in squamous metaplasia (Fig. 4) which may extend into the submucosal gland ducts. Necrotic cartilages may also show evidence of attempted repair, with the development of new tissue around (but not in) the original necrotic cartilage. In other cases, the cartilage may undergo mineralization, often accompanied by a granulomatous reaction.

Changes in the submucosal glands are rare, but may develop under 2 circumstances. In some cases, the development of a squamous metaplastic epithelium, particularly when keratinized, appears to “seal over” the gland duct opening. This duct obstruction initially leads to cystic distension and subsequently to acinar atrophy. In addition, a similar lesion may result in cases of severe ulceration and exudation, the exudate itself obstructing the duct leading to the build up of mucus.

As mentioned earlier, few compound specific lesions have been reported in the larynx and most compounds induce variations of the changes described above. However, a few more specific lesions have been described in this laboratory, e.g., agrochemical induced ulcerative lesions solely on the outer aspects of the arytenoid projections rather than on the more usual inner aspects. Inflammatory polyps have been described following exposure to cobalt sulfate heptahydrate aerosols (4). These were located at the base of the epiglottis. Laryngeal granulomas were found in rats exposed to an aqueous silane solution (8).

The reason(s) for the susceptibility of the rat larynx to undergo squamous metaplasia following inhalation of a wide variety of chemicals is uncertain, but probably involves several factors (5, 6, 9), not least of these, is the location of isolated islands of ciliated cells in the middle of squamous regions at the base of the epiglottis, a site more likely to be subjected to inhaled insults than other respiratory areas. Similar changes have also been induced by the inhalation of glycerol and ascorbic acid (5) and, in this laboratory, the addition of 10% alcohol to a previously inert pharmaceutical vehicle. Although most of the work in this laboratory is performed on rats, a small number of experiments with hamsters.
and mice have shown similar results. However, in our experience, administration of pharmaceuticals, previously shown to induce changes in rats, usually fails to result in the development of lesions in primates. Experiments to investigate possible reversibility have generally been inconclusive, often with evidence of partial regression. The development of metaplastic changes inevitably presents interpretative problems with the extrapolation of findings to man, especially with pharmaceuticals. At this point, it would be of great value to be able to conclusively distinguish between “adaptive” squamous metaplasia and “preneoplastic” squamous metaplasia (5). The absence of atypia and dysplasia, the lack of progression even after 2 yr exposure and the usual absence of lesions in primates suggests that the squamous metaplasia is probably only a defence mechanism (“adaptive”) by which a susceptible epithelium is replaced by a more resistant type (6).

REFERENCES


