sustentacular cells has been observed following inhalation of a variety of chemicals (Buckley et al. 1985; Hardisty et al. 1999; Monticello, Morgan, and Uraih 1990).

Degeneration (Figures 5–7): Nasal Cavity

Pathogenesis/cell of origin: Squamous, transitional, respiratory, olfactory, or glandular epithelium.

Diagnostic features:

- Loss of cilia
- Epithelial vacuolization/bleb formation
- Increased intercellular spaces
- Loss of organization of the cell layers
- Dilatation (ectasia) of nasal glands with accumulation of secretory material

Differential diagnoses:

- Postmortem autolysis: Uniform dissolution of entire tissue section with no change in organization or depth of cell layers
- Metaplasia: Change in epithelial cell types present, usually with mixture of cell types in areas of transition
- Atrophy: Thinning of affected mucosa, loss of axon bundles or other adjacent structures
- Tangential section through epithelium: Microscopic evidence of tangential cut in other tissue structures

Comment: Degenerative changes in nasal tissue have been reported as sequelae to toxic agent exposure (Harkema 1990; Monticello, Morgan, and Uraih 1990; Morgan and Harkema 1996) and as a consequence of aging (St. Clair and Morgan 1992). Accessory nasal structures and surrounding tissues can also undergo a variety of degenerative changes. The vomeronasal organ, a specialized sensory organ thought to be responsible for pheromone recognition and food flavor perception, is composed of sensory and columnar epithelia. This organ can undergo degenerative and atrophic changes following exposure to toxic agents or as a consequence of aging (Uraih and Maronpot 1990; Sills, Morgan, and Boorman 1994).

Eosinophilic Globules (Droplets) (Figures 8 and 9): Nasal Cavity

Synonyms: Hyaline droplets; eosinophilic inclusions; hyaline droplet accumulation.

Pathogenesis/cell of origin: Respiratory, glandular, and olfactory epithelium.

Diagnostic features:

- Accumulation of brightly eosinophilic cytoplasmic inclusions in sustentacular cells of olfactory epithelium, respiratory epithelial cells, and epithelial cells of the nasal seromucous glands
- Most prominent near the junction of olfactory and respiratory epithelia

Differential diagnosis:

- Cytoplasmic changes from specific toxic agents

Comment: Eosinophilic inclusions are observed occasionally in otherwise normal epithelium of untreated rats, more frequently in aged animals (Boorman, Morgan, and Uraih 1990; Monticello, Morgan, and Uraih 1990). They may be seen in association with loss of sensory cells. These inclusions are negative for periodic acid-Schiff (PAS), Alcian Blue, Von Kossa, mucicarmine, phosphotungstic acid hematoxyline (PTAH), Masson's trichrome, Congo red, and toluidine blue stains (Monticello, Morgan, and Uraih 1990). Ultrastructurally, they present as amorphorous flocculent (presumably proteinaceous) material in membrane-bound vacuoles. Increases in the incidence and severity of eosinophilic globules in respiratory and olfactory epithelia are frequently observed in inhalation studies and have been observed in rats exposed to dimethylamine (Buckley 1985; Gross, Patterson, and Morgan 1987) or cigarette smoke (J. Lewis, Nikula, and Sachetti 1994). Inclusions in nasal epithelium of smoke-exposed mice reacted with antibodies for carboxylesterase, an enzyme induced by exposure to some toxic compounds, and with antibodies to the Ym1 sequence of the protein Ym2, a member of the chitinase family (Ward et al. 2001).

Corpora Amylacea (Figure 10): Nasal Cavity

Pathogenesis/cell of origin: Olfactory or respiratory epithelium and adjacent lamina propria, lumen of nasal glands.

Diagnostic features:

- Small basophilic or amphophilic concretions
- Often laminar with mineralized areas

Differential diagnoses:

- Necrosis: Other evidence of tissue damage
- Degeneration: Other evidence of tissue damage
- Nasal exudate or foreign bodies: other evidence of exudate or foreign body
- Mineralization: Evidence of mineralization at other sites or tissues

Comment: Corpora amylacea are seen infrequently in untreated rats and mice (Monticello, Mogan, and Uraih. 1990) and have been described in mice exposed to dimethylamine (Buckley et al. 1985).

Amyloid (Figure 11): Nasal Cavity

Pathogenesis/cell of origin: Extracellular deposits of polypeptide fragments of a chemically diverse group of glycoproteins (Myers and McGavin 2007) in various tissues, including nasal epithelium.

Diagnostic features:

- Lightly eosinophilic, amorphous extracellular material in submucosa

- Green birefringence using polarized light with Congo Red stain

Differential diagnoses:

- Necrosis: Congo Red negative; other evidence of damage
- Degeneration: Congo Red negative
- Connective tissue hyaline: Congo Red negative
- Nasal exudate or foreign bodies: Congo Red negative; other evidence of exudate or foreign body

Comment: Amyloid and amyloid-like materials have been observed in various tissues of aged mice of several strains, including the nasal cavity (Herbert and Leininger 1999; Haines, Chattopadhyay, and Ward 2001; Korenaga et al. 2004).

Necrosis (Figures 1, 12–15): Nasal Cavity

Pathogenesis/cell of origin: Squamous, transitional, respiratory, olfactory, or glandular epithelium, turbinates.

Diagnostic features:

- Pyknosis or karyorrhexis of nuclei
- Cytoplasmic eosinophilia
- Cellular swelling or shrinkage
- Exfoliation of cells
- May result in erosion or ulceration
- May be associated with inflammation
- Luminal accumulations of fibrin and/or cell debris

Differential diagnoses:

- Postmortem autolysis: Uniform dissolution of entire tissue section with no change in organization or depth of cell layers
- Degeneration: Loss of cilia and vacuolation but no inflammation or cell debris
- Atrophy: Thinning of cell layers but no inflammation or cell debris
- Inflammation: Cellular infiltrates, congestion, and/or edema but no exfoliation or cell debris

Comment: Respiratory or transitional epithelium lining the dorsal medial, middle medial, and lateral meatuses are often the first areas affected after exposure to irritant gases. The subsequent inflammatory response with exudation of fibrin and cellular debris may set the stage for adhesion of turbinates to adjacent structures. The area of olfactory epithelium most frequently affected by inhalation of direct-acting gaseous irritants is the most rostral olfactory epithelium lining the dorsal medial meatus (Buckley et al. 1984; Hardisty et al. 1999). Chemicals requiring metabolism to a toxic intermediate to damage olfactory epithelium usually induce lesions throughout the olfactory epithelium (Gaskell 1990) and may also induce necrosis in adjacent Bowman's glands. A frequent sequela to loss of sensory cells (either through aging or toxic injury) is atrophy of nerve bundles within the lamina propria. Conversely, surgical transection of axons or therapy with antimicrotubule drugs induces apoptosis of olfactory epithelium (Levin et al. 1999; Kai et al. 2004). Toxic injury may affect the nasal glands, with subsequent loss of epithelium and replacement with fibrous connective tissue. This may also occur spontaneously, perhaps as an aging phenomenon. Extensive necrosis may also involve the underlying bone of the nasal turbinates and may lead to septal perforation.

Erosion/Ulceration (Figures 16–18): Nasal Cavity

Pathogenesis/cell of origin: Squamous, transitional, respiratory, olfactory, or glandular epithelium.

Diagnostic features:

- Loss of nasal epithelium only (erosion)
- Complete loss of the epithelium and underlying basement membrane (ulceration)
- Associated with necrosis and inflammation, usually suppurative or serofibrinous

Differential diagnoses:

- Artifact: No evidence of inflammation
- Atrophy: Thinning of mucosa but no inflammation or cell debris
- Autolysis: Uniform dissolution of entire tissue section with no change in organization or depth of cell layers
- Necrosis: Cellular features of necrosis (see above) but mucosa is intact
- Degeneration: Cellular features of degeneration (see above) but mucosa is intact

Comment: Erosion and ulceration are observed in all types of nasal epithelium in response to inhaled toxicants (Monticello, Morgan, and Uraih 1990). Care must be taken to distinguish erosion or ulceration from artifactual loss of epithelium related to experimental procedures or autolysis. A serofibrinous or suppurative exudate is usually visible covering areas of ulcerated epithelium. Metaplasia to squamous or respiratory epithelium may occur following repeated loss of respiratory or olfactory epithelium, respectively. When ulceration is present as part of a necrotic process, recording of both necrosis and ulceration will provide a clear description of the lesion.

Regeneration (Figures 12 and 19): Nasal Cavity

Pathogenesis/cell of origin: Squamous, transitional, respiratory, olfactory, or glandular epithelium.

Diagnostic features:

- Normal appearing epithelial cells with basophilic cytoplasm
- Increased nuclear:cytoplasmic ratio
- Epithelial architecture may remain irregular
- Adjacent to or within areas of degenerating, necrotic, hyperplastic, or metaplastic epithelium

Differential diagnoses:

- Hyperplasia: Epithelium is thickened due to increased numbers of cells, resulting in undulating, rugose epithelial surface and irregular arrangement of cell layers (see proliferative lesion section of this document)
- Neoplasia: Expansile nodule is usually protruding into cavity, with cellular atypia and compression of adjacent structures (see proliferative lesion section of this document)

Comment: Regeneration is a term indicating the growth of cells and tissues to replace lost or damaged structures, as opposed to hyperplasia, a term denoting an increase in the number of cells beyond normal in a tissue (Kumar, Abbas, and Fausto 2004). Irregularity of epithelial cell arrangement is often observed in the olfactory epithelium in the process of regeneration. Degeneration, necrosis, and regeneration are often present together in nasal epithelium repeatedly injured by toxicants. See comments above in sections on degeneration and necrosis.

C. Inflammation, Nasal Cavity (Synonym: Rhinitis)

The nasal mucosa is a well-known site for chemically induced injury. The tips of the maxillary and nasal turbinates are frequently the areas first and most severely affected by inhaled irritants. Associated nasal cavity structures (e.g., nasolacrimal duct) may also be affected by inflammatory processes. Foreign bodies, generally either food particles or bedding material, are occasionally found in the nasal cavity. Foreign bodies in nasal cavity tissues elicit an initial suppurative inflammatory response, accompanied or followed by influx of macrophages. If the foreign body persists, the inflammatory response will gradually evolve to chronic inflammation dominated by macrophages with varying numbers of other cell types. Fibrosis may also be present.

Inflammation, Acute (Figures 4 and 20): Nasal Cavity
Pathogenesis/cell of origin: Squamous, transitional, respiratory, olfactory, or glandular epithelium and associated tissues and cavities.

Diagnostic features:

- Vascular congestion
- Edema
- Accumulation of serous, mucous, or fibrinous exudates
- Neutrophils

Differential diagnoses:

- Necrosis: Pyknosis, karyorrhexis of nuclei, cytoplasmic swelling or shrinkage, cellular debris, accompanied by inflammatory infiltrate
- Chronic active inflammation: Cellular infiltrate is a mixture of granulocytic, lymphocytic, histiocytic cells, fibrosis

Comment: Migration of neutrophils into nasal passages produces a suppurative exudate. Eosinophils in exudate or mucosal infiltrate indicate an immunologic element to the inflammatory process (Ibanes et al. 1996).

Inflammation, Chronic: Nasal Cavity
Pathogenesis/cell of origin: Squamous, transitional, respiratory, olfactory, or glandular epithelium and associated tissues and cavities.

Diagnostic features:

- Cellular infiltrate is predominantly lymphocytes, plasma cells, and macrophages
- Hyperplasia of affected epithelium and fibroplasia may be present

Differential diagnoses:

- Other types of inflammation (see below)
- Early connective tissue or hematopoietic neoplasia

Comment: Chronic inflammation may have different characteristics depending on the duration of the lesion and the initiating cause.

Inflammation, Chronic Active (Figures 21 and 22): Nasal Cavity
Pathogenesis/cell of origin: Squamous, transitional, respiratory, olfactory, or glandular epithelium and associated tissues and cavities.

Diagnostic features:

- Cellular infiltrate is clearly a mixture of granulocytic cells with lymphocytic and/or histiocytic cell types
- Congestion, edema, mucous exudates, or other evidence of acute inflammation may be present
- Fibroplasia and hyperplasia or metaplasia of affected epithelium may be present

Differential diagnoses:

- Other types of inflammation (see below)
- Early connective tissue or hematopoietic neoplasia

Comment: The term *chronic active inflammation* implies recurrence or persistence of granulocytic inflammatory cells concurrent with ongoing chronic inflammation. Chronic active and granulomatous inflammation (see below) have many etiologic and morphologic similarities.

Inflammation, Granulomatous (Figures 23–30): Nasal Cavity
Pathogenesis/cell of origin: Squamous, transitional, respiratory, olfactory, or glandular epithelium and associated tissues and cavities.

Diagnostic features:

- Cellular infiltrate is predominantly plump macrophages (epithelioid cells), which may form interlacing bundles, accompanied by lymphocytes, plasma cells, and fibrosis, depending on duration and etiologic agent
- Infiltrating macrophages may form multinucleated giant cells
- Etiologic agent may be visible, for example, fungi, mycobacteria, or foreign bodies
- Granulocytes may be present in the affected area, in which case the process could be described as pyogranulomatous inflammation
- Hyperplasia or metaplasia of affected epithelium may be present

Differential diagnoses:

- Other types of inflammation (see below)
- Early connective tissue or hematopoietic neoplasia

Comment: Granulomatous inflammation suggests an etiologic agent that is resistant to dissolution or is immunogenic, for example, fungi, mycobacteria, or foreign body (Kumar, Abbas, and Fausto 2004). Foreign body inflammation owing to deposition of animal hairs is observed occasionally in untreated aged rats (Nagano et al. 1997; Takeuchi, Nagano, Aiso et al. 1997). The lesion occurs in the anterior, median, and posterior meatuses, most frequently in the medial meatus. Enhancement of the foreign body inflammation as a result of inhalation of a chemical was reported in a chronic study (Takeuchi, Nagano, Katagiri et al. 1997). It is important to distinguish foreign body inflammation from other inflammatory lesions, because of the differences in their causes. Inflammatory polyps consisting of proliferating submucosal connective tissue lined by metaplastic, hyperplastic, or normal mucosal epithelium (Figure 27), related to dental dysplasia, are reported to occur in the nasal cavity of mice (Losco 1995), and secondary to squamous metaplasia in the larynx of rats (Bucher et al. 1990).

Inflammation, Lymphocytic or Eosinophilic: Nasal Cavity

Pathogenesis/cell of origin: Squamous, transitional, respiratory, olfactory, or glandular epithelium and associated tissues and cavities.

Diagnostic features:

- Infiltration of a relatively pure population of lymphocytes, plasma cells, or eosinophils into the lamina propria of the nasal cavity and associated tissues

Differential diagnoses:

- Acute inflammation: Vascular congestion, edema, serous, or mucous exudate, few neutrophils present
- Other types of chronic inflammation (see above)
- Hyperplasia of nasal-associated lymphoid tissue (NALT): Localized submucosal nodule of lymphocytes not infiltrating cavities and limited to adjacent epithelium
- Hematopoietic neoplasia: Homogenous lymphocyte population infiltrating entire tissue and other sites

Comment: Inflammation consisting only of lymphocytes or plasma cells may indicate an immunologic basis to the inflammation or may represent lymphoid hyperplasia rather than inflammation. Nasal-associated lymphoid tissue adjacent to the nasopharyngeal duct of rodents and other laboratory animal species has characteristics and functions similar to those of other mucosal-associated lymphoid tissues and is the subject of immunological research (Harkema, Carey, and Wagner 2006). Nasal-associated lymphoid tissue has been recommended as a tissue to be examined routinely in inhalation toxicology studies (Renne et al. 2007). Eosinophilic infiltrates may indicate an immunologic basis to the inflammation, including that incited by parasites or allergic reactions.

D. Vascular Changes, Nasal Cavity

Congestion: Nasal Cavity

Synonym: Hyperemia.

Pathogenesis/cell of origin: Vasculature of nasal tissues and surrounding tissue.

Diagnostic features:

- Widely dilated, blood-filled submucosal vessels

Differential diagnoses:

- Postmortem autolysis: Uniform dissolution of entire tissue section with lysis of RBCs
- Angiectasis: Dilated vessels that distort normal architecture of the affected tissues

Comment: Congestion of the richly vascular nasal mucosa may be observed in rats dying or killed while moribund and is related to terminal pooling of blood in the nasal cavity.

Edema: Nasal Cavity

Pathogenesis/cell of origin: Vasculature of nasal tissues and surrounding tissue.

Diagnostic features:

- Proteinaceous fluid around vessels and free in lumen of nasal cavity

Differential diagnoses:

- Postmortem autolysis: Uniform dissolution of entire tissue section with lysis of RBCs
- Fibrinous exudate: Smudged pink exudate with laminar appearance of fibrils visible at high magnification

Comment: Hyperemia, edema, and/or hemorrhage in the nasal mucosa may be the earliest response observed to inhaled nasal toxicants or traumatic injury to the nose.

Hemorrhage: Nasal Cavity
Pathogenesis/cell of origin: Lumen and nasal cavity tissues.
Diagnostic features:

- Presence of extravascular red blood cells in the nasal passages or tissues

Differential diagnosis:

- Iatrogenic bleeding resulting from blood collection via orbital sinus
- Angiectasis: Blood present within dilated vascular lumens

Comment: Small amounts of blood may be observed in the nasolacrimal duct or nasal passages following antemortem blood collection via the retroorbital plexus in the medial canthus of the eye. Hemorrhage in the nasal cavity may also be a result of trauma, infection, or inhaled irritant chemicals and noxious gases or vapors.

Angiectasis: Nasal Cavity
Pathogenesis/cell of origin: Vasculature of nasal tissues and surrounding tissue.
Diagnostic features:

- Increased profiles of blood vessels, distorting the normal architecture of the affected tissue

Differential diagnoses:

- Hemangioma: Focal blood-filled spaces lined with uniform endothelial cells, distorting the architecture of the affected tissue
- Congestion: Widely dilated, blood-filled vasculature not distorting the architecture of the affected tissue
- Hemorrhage: Extravascular blood present in the nasal passages or tissues

Comment: Angiectasis and/or thrombosis may be seen in the nasal septum, turbinates, or lateral wall and are usually associated with mononuclear cell leukemia or a generalized debilitated condition (Boorman, Morgan, and Uraih 1990).

Thrombosis (Figure 31): Nasal Cavity
Pathogenesis/cell of origin: Vessels of nasal submucosa and associated tissues.
Diagnostic features:

- Amorphous pink/gray, clearly laminated mass containing leucocytes and erythrocytes
- Attachment to lumen of vessel is rarely visible on routine sections

Differential diagnosis:

- Postmortem clot: Few or no leucocytes; lamination absent or very fine filaments

Comment: Thrombi are also commonly associated with mononuclear cell leukemia in rats or generalized debilitation.

E. Nonneoplastic Proliferative Lesions: Nasal Cavity

Metaplasia, Squamous Cell (Figure 32): Nasal Cavity, Nasopharynx, Paranasal Sinus
Pathogenesis/cell of origin: Metaplasia of transitional, respiratory, or olfactory epithelia. May also occur in the nasolacrimal ducts and ducts of glands in the lamina propria.
Diagnostic features:

- Characterized by replacement of transitional, respiratory, olfactory, or ductal epithelium by squamous epithelium
- Several layers of stratified epithelial cells, with flattening of the more superficial cells
- Compact or large epithelial cells with complete loss of cilia
- Deeper nuclei are mostly round to oval, with a distinct nucleolus; superficial nuclei more flattened
- Sometimes slight nuclear polymorphism and cellular atypia
- Surface cells might contain only keratohyaline granules, or they might be excessively keratinized
- Often desquamation of surface cells

Differential diagnoses:

- Papilloma, squamous cell: A papillary or filiform projection above the surface of the epithelium or extension into the lumen of the ducts of the submucosal glands. There is delicate vascular mesenchymal stroma and marked proliferative epithelial thickening
- Carcinoma, squamous cell: Characterized by destruction of the basement membrane; cellular atypia and disorientation; frequent mitoses; or presence of other signs of malignancy, such as invasive growth or metastases
- Regeneration: Usually follows acute injury. Cells are one or possibly two layers thick, with increased basophilia; slight karyomegaly, but there is no horizontal layering (stratification) of flattened cells as in squamous metaplasia

Comment: Squamous metaplasia sometimes occurs in association with chronic inflammation or in the process of

regeneration. Squamous metaplasia with a normal maturation pattern may be reversible under some experimental circumstances (e.g., depending on nature of inhaled irritant and duration of exposure) but in other situations may eventually give rise to squamous cell papilloma or squamous cell carcinoma.

(Belinsky et al. 1987; Boorman, Morgan, and Uraih 1990; Brown 1990; Brown et al. 1991; Dungworth, Ernst, et al. 1992; Dungworth et al. 2001; Feron, Woutersen, and Spit 1986; Gopinath, Prentice, and Lewis 1987; Harkema 1990; Holmström, Wilhelmsson, and Hellquist 1989; Jiang, Buckley, and Morgan 1983; Jiang, Morgan, and Beauchamp 1986; Kerns et al. 1983; Maronpot et al. 1986; Monticello, Morgan, and Uraih 1990; Quest et al. 1984; Renne et al. 1986; Reznik, Stinson, and Ward 1980; Reznik-Schüller 1983a; Reznik-Schüller 1983b; Rivenson et al. 1983; Schüller, Gregg, and Reznik 1990; Takahashi, Iwasaki, and Ide 1985; Turk, Henk, and Flory 1987; Yu et al. 1989).

Metaplasia, Respiratory, Olfactory/Glandular Epithelium, Nasal Cavity (Figure 33):

Pathogenesis/cell of origin: Metaplasia of cells of olfactory epithelium and/or cells of submucosal glands in the lamina propria.

Diagnostic features:

- Respiratory epithelial metaplasia of olfactory epithelium is most common in the epithelium lining the dorsal medial meatus
- Characterized by loss of sensory and sustentacular neuroepithelial cells, which may be associated with focal atrophy and degeneration
- Replacement is by ciliated or nonciliated simple columnar epithelium that resembles respiratory epithelium
- Frequently, respiratory epithelial metaplasia extends into the submucosal glands adjacent to the affected segments of olfactory epithelium

Differential diagnoses:

- Hyperplasia, olfactory epithelium: Increased thickness of the epithelium owing to proliferation of sustentacular, precursor olfactory sensory, and/or basal cells. Proliferating cells are disorganized and usually not ciliated
- Precise anatomic localization and comparison with controls are necessary to ensure that the site should normally be lined by olfactory epithelium

Comment: This change is occasionally seen spontaneously in aged rats and mice or may be a response to an irritant. The spontaneous lesion tends to affect one side more than the other and is associated with slightly dilated Bowman's glands, possibly containing neutrophils and/or eosinophilic granular debris. There is no evidence that respiratory epithelial metaplasia is preneoplastic.The normal extension of olfactory epithelium into the anterior region of the dorsal meatus may vary with age and can be possibly strain dependent.

(Belinsky et al. 1987; Boorman, Morgan, and Uraih 1990; Brown 1990; Dungworth, Ernst, et al. 1992; Dungworth et al. 2001; Gaskell 1990; Jiang, Morgan, and Beauchamp 1986; Monticello, Morgan, and Uraih 1990; Morgan 1991; Nagano et al. 1988)

Hyperplasia, Squamous Cell (Figure 34): Nasal Cavity

Pathogenesis/cell of origin: Proliferation of squamous epithelium of the nasal vestibule or ventral meatus, or of metaplastic squamous epithelium.

Diagnostic features:

- Occurs in the squamous epithelium of the nasal vestibule or ventral meatus
- Focal increase in the number of cells to five or more epithelial cell layers
- Normal differentiation pattern
- Occasional mitoses are observed
- The hyperplastic squamous cells have larger nuclei, more prominent nucleoli, and more abundant cytoplasm

Differential diagnoses:

- Papilloma, squamous cell: An exophytic growth of squamous cells, sometimes mixed with cuboidal or mucus-producing cells, resting on a vascularized stalk of connective tissue
- Squamous or adenosquamous carcinoma: Epithelial hyperplasia with cellular atypia might progress to squamous or adenosquamous carcinoma. Diagnosis of a malignant tumor is based on one or more features including overall size, loss of polarity of epithelium, a high degree of atypia, increased numbers of mitoses, or invasive behavior

Comment: Since the thickness of the epithelium varies slightly with location within the nasal vestibule, careful comparison to controls is important for the identification of mild squamous cell hyperplasia.

(Belinsky et al. 1987; Boorman, Morgan, and Uraih 1990; Brown 1990; Brown et al. 1991; Buckley et al. 1985; Dungworth, Hahn et al. 1992; Dungworth et al. 2001; Feron, Woutersen, and Spit 1986; Gaskell 1990; Gopinath, Prentice, and Lewis 1987; Harkema 1990; Jiang, Morgan, and Beauchamp 1986; Maronpot et al. 1986; Monticello, Morgan, and Uraih 1990; Morgan 1991; Quest et al. 1984; Renne et al. 1986; Reznik, Stinson, and Ward 1980; Reznik, Schuller, and Stinson 1994; Reznik-Schüller 1983a, 1983b; Rivenson et al. 1983; Schüller, Gregg, and Reznick 1990; Takahashi, Iwasaki, and Ide 1985; Yamamoto et al. 1990; Yu et al. 1989)

Hyperplasia, Transitional Epithelium (Figure 35): Nasal Cavity

Pathogenesis/cell of origin: Transitional epithelium lining the nasal cavity between the squamous epithelium cranially and the respiratory epithelium caudally.

Diagnostic features:

- Hyperplasia of nonciliated cuboidal/columnar epithelium composed of three or more cell layers

Differential diagnoses:

- Adenoma: Usually an expansile nodular mass that may protrude into the nasal or paranasal cavities. Cellular atypia is common in the case of endophytic growth. Adenoma of the subepithelial glands causes compression of the adjacent tissues and structures
- Papilloma, squamous cell: An exophytic growth of squamous cells, sometimes mixed with cuboidal or mucus-producing cells, resting on a vascularized stalk of connective tissue
- Adenocarcinoma, adenosquamous, or neuroepithelial carcinoma: Epithelial hyperplasia with cellular atypia might progress to adenocarcinoma, adenosquamous carcinoma or neuroepithelial carcinoma (depending on site and principal cell involved). Diagnosis of a malignant tumor is based on one or more features including overall size, loss of polarity of epithelium, a high degree of atypia, increased numbers of mitoses, or invasive behavior

(Belinsky et al. 1987; Boorman, Morgan, and Uraih 1990; Brown 1990; Brown et al. 1991; Buckley et al. 1985; Dungworth et al. 1992; Dungworth et al. 2001; Feron, Woutersen, and Spit 1986; Gaskell 1990; Gopinath, Prentice, and Lewis 1987; Harkema 1990; Jiang, Morgan, and Beauchamp 1986; Maronpot et al. 1986; Monticello, Morgan, and Uraih 1990; Morgan 1991; Quest et al. 1984; Renne et al. 1986; Reznik, Stinson, and Ward 1980; Reznik, Schuller, and Stinson 1994; Reznik-Schüller 1983a; Reznik-Schüller 1983b; Rivenson et al. 1983; Schüller, Gregg, and Reznik 1990; Takahashi, Iwasaki, and Ide 1985; Yamamoto et al. 1990; Yu et al. 1989)

Hyperplasia, Respiratory Epithelium (Figure 36): Nasal Cavity, Nasopharynx, Paranasal Sinus

Pathogenesis/cell of origin: Proliferation of respiratory epithelium.

Diagnostic features:

- Epithelium thickened as a result of an increase in number of basal, mucous, nonciliated cuboidal/columnar, squamous, or olfactory cells
- Epithelial hyperplasia may result in an undulating rugose appearing epithelium
- Ciliated cells may be crowded or less evident owing to the increase in number of nonciliated cells
- Frequently irregular arrangement of cell layers
- Often associated with degeneration and inflammation
- Occasionally increase in the number of cells of subepithelial glands in the nasal septum, maxillo-, naso- and ethmoturbinates
- Epithelial hyperplasia may include proliferation of atypical or pleomorphic basal or undifferentiated cells (epithelial hyperplasia with cellular atypia), but without disruption of the underlying basal lamina

Differential diagnoses:

- Adenoma: Usually an expansile nodular mass that may protrude into the nasal or paranasal cavities. Cellular atypia is common in the case of endophytic growth. Adenoma of the subepithelial glands causes compression of the adjacent structures
- Squamous cell papilloma: An exophytic growth of squamous cells, sometimes mixed with cuboidal or mucus-producing cells, resting on a vascularized stalk of connective tissue
- Adenocarcinoma, adenosquamous, or neuroepithelial carcinoma: Epithelial hyperplasia with cellular atypia might progress to adenocarcinoma, adenosquamous carcinoma or neuroepithelial carcinoma (depending on site and principal cell involved). Diagnosis of a malignant tumor is based on one or more features including overall size, loss of polarity of epithelium, a high degree of atypia, increased numbers of mitoses, and invasive behavior

Comment: Epithelial hyperplasia is frequently a reversible alteration. Areas of epithelial hyperplasia with cellular atypia should not be confused with regenerative responses that follow acute injury to this type of epithelium. Epithelial hyperplasia of transitional epithelium lining the lateral meatus is commonly caused by inhaled irritants. Squamous cell metaplasia may occur within hyperplastic areas. Eosinophilic globules (eosinophilic intracytoplasmic proteinaceous accumulations) may be a prominent feature in all types of epithelial hyperplasia. They may also be found in non-hyperplastic cell populations and are generally considered a degenerative change (dilated endoplasmic reticulum containing proteinaceous material).

(Belinsky et al. 1987; Boorman, Morgan, and Uraih 1990; Brown 1990; Brown et al. 1991; Buckley et al. 1985; Dungworth, Hahn, et al. 1992; Dungworth et al. 2001; Feron, Woutersen, and Spit 1986; Gaskell 1990; Gopinath, Prentice, and Lewis 1987; Harkema 1990; Jiang, Morgan, and Beauchamp 1986; Maronpot et al. 1986; Monticello, Morgan, and Uraih 1990; Morgan 1991; Quest et al. 1984; Renne et al. 1986; Reznik, Stinson, and Ward 1980; Reznik, Schuller, and Stinson 1994; Reznik-Schüller 1983; Rivenson et al. 1983; Schülle, Gregg, and Reznik 1990; Takahashi, Iwasaki, and Ide 1985; Yamamoto et al. 1990; Yu et al. 1989).

Hyperplasia/Metaplasia, Mucous Cell (Figure 37): Nasal Cavity, Nasopharynx, Paranasal Sinus

Synonym(s): Hyperplasia, goblet cell; metaplasia, mucous cell; metaplasia, goblet cell.

Pathogenesis/cell of origin: Basal cells in transitional epithelium or mucous (goblet) cells in respiratory epithelium.

Diagnostic features:

- Increased numbers of mucous cells in transitional and/or respiratory epithelium
- Metaplasia and hyperplasia are distinguished by anatomic location; significant numbers of mucous cells are not normally present in transitional epithelial zone of rodent nasal mucosa.
- Hyperplastic mucous cells in the surface epithelium may form intraepithelial glands.
- Mucous cells are taller, with apical mucous granules and basally located nuclei.

Differential diagnoses:

- Adenoma: An expansile nodular mass that may protrude into the nasal or paranasal cavities. Cellular atypia is common in the case of endophytic growth. Adenoma of the subepithelial glands causes compression of the adjacent structures.
- Squamous cell papilloma: Characterized by exophytic growth of squamous cells sometimes mixed with cuboidal or mucus-producing cells, resting on a vascularized stalk of connective tissue.
- Adenocarcinoma, adenosquamous, or neuroepithelial carcinoma: Epithelial hyperplasia with cellular atypia might progress to adenocarcinoma, adenosquamous carcinoma, or neuroepithelial carcinoma (depending on site and principal cell involved). Diagnosis of a malignant tumor is based on one or more features including overall size, loss of polarity of epithelium, a high degree of atypia, increased numbers of mitoses, or invasive behavior.

Comment: Mucous cell metaplasia and hyperplasia are frequently observed in the anterior nasal cavity of rodents in response to repeated inhalation of irritants. There is no evidence that mucous cell hyperplasia is preneoplastic. It develops from hypertrophic epithelium with typical goblet cells distended with secretory droplets.

(Belinsky et al. 1987; Boorman, Morgan, and Uraih 1990; Brown 1990; Brown et al. 1991; Buckley et al. 1985; Dungworth, Ernst, et al. 1992; Dungworth et al. 2001; Feron, Woutersen, and Spit 1986; Gaskell 1990; Gopinath, Prentice, and Lewis 1987; Harkema 1990; Jiang, Morgan, and Beauchamp 1986; Maronpot et al. 1986; Monticello, Morgan, and Uraih 1990; Morgan 1991; Quest et al. 1984; Renne et al. 1986; Reznik, Stinson, and Ward 1980; Reznik, Schuller, and Stinson 1994; Reznik-Schüller 1983a, 1983b; Rivenson et al. 1983; Schüller, Gregg, and Reznik 1990; Takahashi, Iwasaki, and Ide 1985; Yamamoto et al. 1990; Yu et al. 1989).

Hyperplasia, Olfactory Epithelium (Figure 38): Nasal Cavity

Pathogenesis/cell of origin: Proliferation of precursors of olfactory sensory neurons, sustentacular cells, or basal cells.

Diagnostic features:

- Hyperplasia in olfactory epithelium is characterized by an increased thickness of the epithelium resulting from an increase in the number of sustentacular, olfactory sensory precursor, and/or basal cells

Differential diagnoses:

- Adenoma: An expansile nodular mass that may protrude into the nasal or paranasal cavities. Cellular atypia is common in the case of endophytic growth. Adenoma of the subepithelial glands causes compression of the adjacent structures.
- Squamous cell papilloma: An exophytic growth of squamous cells, sometimes mixed with cuboidal or mucus-producing cells, resting on a vascularized stalk of connective tissue.
- Adenocarcinoma, adenosquamous, or neuroepithelial carcinoma: Epithelial hyperplasia with cellular atypia might progress to adenocarcinoma, adenosquamous carcinoma or neuroepithelial carcinoma (depending on site and principal cell involved). Diagnosis of a malignant tumor is based on one or more features including overall size, loss of polarity of epithelium, a high degree of atypia, increased numbers of mitoses, or invasive behavior.

(Belinsky et al. 1987; Boorman, Morgan, and Uraih 1990; Brown 1990; Brown et al. 1991; Buckley et al. 1985; Dungworth, Hahn et al. 1992; Dungworth et al. 2001; Feron, Woutersen, and Spit 1986; Gaskell 1990; Gopinath, Prentice, and Lewis 1987; Harkema 1990; Jiang, Morgan, and Beauchamp. 1986; Maronpot et al. 1986; Monticello, Morgan, and Uraih 1990; Morgan 1991; Quest et al. 1984; Renne et al. 1986; Reznik, Stinson, and Ward 1980; Reznik, Schuller, and Stinson 1994; Reznik-Schüller 1983a; Reznik-Schüller 1983b; Rivenson et al. 1983; Schüller, Gregg, and Reznik 1990; Takahashi, Iwasaki, and Ide 1985; Yamamoto et al. 1990; Yu et al. 1989).

Hyperplasia, Basal Cell (Figure 39): Nasal Cavity, Nasopharynx, Paranasal Sinus

Pathogenesis/cell of origin: Proliferation of basal cells of respiratory, transitional, olfactory, or squamous epithelium or subepithelial glandular epithelium.

Diagnostic features:

- Density of the basal cells and thickness of the basal cell layer increased
- Usually focal, multifocal, or segmental and may frequently seen to form small nodular or irregular thickening of the basal epithelium

Differential diagnoses:

- Adenoma: An expansile nodular mass that may protrude into the nasal or paranasal cavities. Cellular atypia is common in the case of endophytic growth. Adenoma of the subepithelial glands causes compression of the adjacent structures.
- Squamous cell papilloma: An exophytic growth of squamous cells, sometimes mixed with cuboidal or mucus-producing cells, resting on a vascularized stalk of connective tissue.
- Adenocarcinoma, adenosquamous, or neuroepithelial carcinoma: Epithelial hyperplasia with cellular atypia might progress to adenocarcinoma, adenosquamous carcinoma or neuroepithelial carcinoma (depending on site and principal cell involved). Diagnosis of a malignant tumor is based on one or more features including overall size, loss of polarity of epithelium, a high degree of atypia, increased numbers of mitoses, or invasive behavior.

(Belinsky et al. 1987; Boorman, Morgan, and Uraih 1990; Brown 1990; Brown et al. 1991; Buckley et al. 1985; Dungworth et al. 1992;a Dungworth et al. 2001; Feron, Woutersen, and Spit 1986; Gaskell 1990; Gopinath, Prentice, and Lewis 1987; Harkema 1990; Jiang, Morgan, and Beauchamp. 1986; Maronpot et al. 1986; Monticello, Morgan, and Uraih 1990; Morgan 1991; Quest et al. 1984; Renne et al. 1986; Reznik, Stinson, and Ward 1980; Reznik, Schuller, and Stinson 1994; Reznik-Schüller 1983; Rivenson et al. 1983; Schüller, Gregg, and Reznik 1990; Takahashi, Iwasaki, and Ide 1985; Yamamoto et al. 1990; Yu et al. 1989).

Hyperplasia, Neuroendocrine Cell: Nasal Cavity, Nasopharynx, Paranasal Sinus

Pathogenesis/cell of origin: Proliferative growth of clusters of neuroendocrine cells within the respiratory or olfactory epithelium.

Diagnostic features:

- Clusters of uniform small cells within the olfactory epithelium or may appear to arise from the basal epithelium. Cells have scant often basophilic cytoplasm and nuclei with stippled chromatin.
- Definitive identification of hyperplastic neuroendocrine cells requires immunostaining for specific markers. For the mouse, the best markers appear to be protein gene product 9.5 (PGP), calcitonin, calcitonin gene-related peptide (CGRP), and helodermin.

Differential diagnosis:

- Carcinoma, neuroepithelial: Neuroendocrine cell hyperplasia might progress to neuroepithelial carcinoma. Diagnosis of a malignant tumor is based on one or more features including overall size, loss of polarity of epithelium, a high degree of atypia, increased numbers of mitoses, or invasive behavior.

(Haworth et al. 2007; Kasacka and Sawicki 2004; Levasseur et al. 2004; Reznik, Stinson, and Ward 1980; Reznik-Schüller 1983a, 1983b; Rouquier and Giorgi 2007; Schüller, Gregg, and Reznik 1990; Shimosegawa and Said 1991; Trinh and Storm 2004)

Hyperplasia with Atypia (Figure 40): Nasal Cavity, Nasopharynx, Paranasal Sinus

Pathogenesis/cell of origin: Hyperplastic squamous, respiratory, olfactory, or glandular epithelium.

Diagnostic features:

- Proliferating epithelium contains many basal or undifferentiated cells
- Numerous cells with increased nuclear: cytoplasmic ratio, atypical shape or size
- Atypical cells extend into adjacent glands

Differential diagnosis:

- Carcinoma: Presence of signs of malignancy such as invasion of or extension into adjacent tissue or metastases

F. Neoplastic Proliferative Lesions, Nasal Cavity

Papilloma, Squamous Cell (Figure 41): Nasal Cavity, Nasopharynx, Paranasal Sinus

Pathogenesis/cell of origin: May originate from transitional, respiratory or metaplastic olfactory epithelium, or from the squamous epithelium of the nasal vestibule.

Diagnostic features:

- Usually exophytic mass of uniform, regularly arranged squamous cells that form papillary or filiform structures
- Epithelial cells cover vascularized stalk of connective tissue
- Intact basement membrane
- Occasionally growth beneath the mucosal surface ("inverted" or "endophytic" papilloma)
- Lesion is covered by squamous surface cells, which may contain only keratohyaline granules or are highly keratinized.

Differential diagnoses:

- Hyperplasia or metaplasia, squamous cell: Usually multifocal and bilateral. No or minimal exophytic growth, does not form papillary projections. No vascularized stalk of connective tissue
- Adenoma: No or few squamous cell areas
- Carcinoma, squamous cell: Destruction of the basement membrane, cellular atypia or disorientation,

frequent mitoses, or presence of other signs of malignancy such as invasive growth or metastases

Comment: Transitional and respiratory epithelial cells may be sometimes observed as a minor component (Boorman, Morgan, and Uraih 1990; Brown 1990; Brown et al. 1991; Dungworth et al. 1992; Dungworth et al. 2001; Laskin et al. 1980; Lee and Trochimowicz 1982; Pour et al. 1976; Pour and Götz 1983; Renne et al. 1986; Reznik, Schuller, and Stinson 1994; Reznik-Schüller 1983; Schüller et al. 1990; Stinson 1983).

Adenoma (Figure 42): Nasal Cavity, Nasopharynx, Paranasal Sinus

Pathogenesis/cell of origin: Neoplastic transformation of transitional, respiratory, or glandular epithelial cells.

Diagnostic features:

- Usually arises in the most anterior part of the nasal cavity, originating from the mucosa of the naso- or maxilloturbinates or from the lateral wall of the anterior nasal cavity
- An expansile growth with occasional protrusion into the nasal or paranasal cavities, but may show endophytic growth as well
- Compression may be caused by endophytic adenomas of respiratory epithelium or adenomas of submucosal glands.
- Glandular structures or sheets of cells, sometimes with pseudoacinar structures resulting from dropout of dead cells. Glandular structures may be cystic
- Cystic glands may contain PAS-positive material, sloughed epithelium, and inflammatory cells.
- Secretory activity is mostly visible as mucus production.
- Usually composed of nonciliated cuboidal to low columnar cells
- Basophilic cytoplasm and centrally located nuclei
- Occasionally focal areas of squamous cell metaplasia
- Epithelial cells may contain eosinophilic globules.

Differential diagnoses:

- Hyperplasia, respiratory epithelial: No exophytic growth. No nodular lesion. Absence of compression of the adjacent tissue.
- Papilloma, squamous cell: Predominantly squamous cell proliferation. Exophytic, mostly papillary or filiform growth. Vascularized stalk of connective tissue.
- Adenocarcinoma: Evidence of malignancy such as cellular or nuclear atypia; frequent mitoses; invasive growth; or metastases

Comment: Adenomas with exophytic growth are sometimes called polypoid or villous adenomas. Location of the tumor usually determines the type of adenoma (transitional or respiratory epithelial type). Transitional epithelial cell tumors are found mostly in the lateral meatus of the anterior (proximal) aspect of the nasal cavity. In many cases, it can be difficult to determine by light microscopy whether the cell of origin is of transitional, respiratory, or submucosal glandular epithelium. Ultrastructurally, adenomas of the glands may show periacinar myoepithelial cells and large apical secretory granules, as demonstrable in normal submucosal glands. Adenomas might arise from glandular epithelium in the lamina propria, although this has not been reported. Eosinophilic globules or droplets (eosinophilic intracytoplasmic proteinaceous accumulations) may be a prominent feature of adenomas. They are generally considered to be dilated endoplasmic reticulum containing proteinaceous material) and occur more frequently in mice than in rats. More information and an illustration of eosinophilic globules in nasal epithelium are presented above in the nonproliferative lesion section of this document.

(Belinsky et al. 1987; Boorman, Morgan, and Uraih 1990; Brown 1990; Brown et al. 1991; Dungworth, Ernst, et al. 1992; Dungworth, Hahn, et al. 1992; Dungworth et al. 2001; Griciute et al. 1986; Kerns 1985a, 1985b; Kerns et al. 1983; Klaassen, Jap, and Kuijpers 1982; Lee and Trochimowicz 1982; Maronpot 1990; R. R. Miller et al. 1985; Renne et al. 1986; Reznik, Schuller, and Stinson 1994; Reznik-Schüller 1983a, 1983b; Schüller, Gregg, and Reznik 1990; St. Clair and Morgan 1992; Stinson 1983; Woutersen et al. 1989).

Carcinoma, Squamous Cell (Figure 43): Nasal Cavity, Nasopharynx, Paranasal Sinus

Pathogenesis/cell of origin: May originate from squamous differentiation of transitional, respiratory, or olfactory epithelial cells; epithelial cells of the subepithelial glands; or from malignant transformation of the squamous epithelium of the nasal vestibule.

Diagnostic features:

- Most often in the anterior nasal cavity, arising from the epithelium of the lateral walls, septum, naso- and maxilloturbinates, and in the ethmoturbinates
- Composed of solid, often branching, cords or masses of cells with various degrees of anaplasia
- Shape and size of cells are irregular: large and polygonal, or flattened and stratified
- Cytoplasm eosinophilic and granular to hyalinized as a result of the high keratin content. Cells may contain only keratohyaline granules or form keratin pearls
- Presence of signs of malignancy such as frequent mitoses, cellular or nuclear atypia, or invasion into surrounding tissues
- Well-differentiated squamous cell carcinomas are predominantly composed of cells that have prominent intercellular bridges, normal keratinization, minimal nuclear atypia, and a low mitotic index.
- Poorly differentiated squamous cell carcinomas have cells with few intercellular bridges, abnormal keratinization (dyskeratosis), nuclear and cytoplasmic

atypia, and abnormal mitotic figures. Spindle-shaped cells might be prominent.

Special techniques for diagnosis:

- Ultrastructural (tonofilaments) or immunohistochemical (keratins) verification might be necessary to determine the origin of poorly differentiated tumors

Differential diagnoses:

- Hyperplasia, squamous epithelium or squamous metaplasia, respiratory, or olfactory epithelium: Absence of characteristics of malignancy such as penetration of the basement membrane, invasion into surrounding tissues, lymphatics, vessels, bronchi, or metastases. Minimal cellular atypia and/or disorganization may occur
- Adenosquamous carcinoma: There may be difficulty in distinguishing adenosquamous carcinoma from squamous cell carcinomas with entrapped submucosal glands. The glandular component of squamous cell carcinomas appears normal, whereas the glandular component of adenosquamous carcinomas has malignant features, for example, frequent mitoses, or cellular or nuclear atypia, or invasion into surrounding tissues, lymphatics, or vessels

Comment: A poorly differentiated, nonkeratinizing squamous cell carcinoma might be misdiagnosed as a poorly differentiated adenocarcinoma. In general, poorly differentiated tumors of the nasal cavity are difficult to classify. Electron microscopy and/or immunohistochemistry may aid in attempting definitive diagnosis.

(Bermudez et al. 1992; Boorman, Morgan, and Uraih 1990; Brown 1990; Brown et al. 1991; Dungworth, Ernst, et al. 1992; Dungworth, Hahn, et al. 1992; Dungworth et al. 2001; Hayashi, Mori, and Nonomaya 1998; Kerns 1985a, 1985b; Kerns et al. 1983; Kociba et al. 1974; Kristiansen et al. 1993; Laskin et al. 1980; Lee and Trochimowicz 1982; Maita et al. 1988; Renne et al. 1986; Reznik, Schuller, and Stinson 1994; Reznik-Schüller 1983a, 1983b; Schüller, Gregg, and Reznik 1990; St. Clair and Morgan 1992; Stinson 1983; Stinson and Reznik-Schüller 1985; Takahashi, Iwasaki, and Ide 1985; Woutersen et al. 1989; Yu et al. 1989).

Carcinoma, Adenosquamous (Figure 44): Nasal Cavity, Nasopharynx, Paranasal Sinus

Pathogenesis/cell of origin: Malignant transformation of respiratory epithelium, submucosal gland duct epithelium, basal and sustentacular cells of the olfactory epithelium, or from areas of metaplasia in the epithelia.

Diagnostic features:

- Tumor has malignant glandular and squamous epithelia components.
- Squamous components of the tumors may show typical keratin pearl formation.
- Tumor may contain undifferentiated epithelial cells
- Presence of malignancy such as frequent mitoses, cellular or nuclear atypia, or invasion into surrounding tissues, lymphatics, vessels, or metastases

Differential diagnoses:

- Papilloma, squamous cell: Composed of a mixture of squamous and mucus-producing cells (mucoepidermoid type), but evidence of malignancy such as marked cytologic atypia, penetration of the basement membrane, invasive growth, or metastases are absent. Squamous proportions predominate in squamous cell papilloma.
- Adenocarcinoma: In general, adenocarcinomas lack a squamous component. Areas of squamous cell metaplasia within an adenocarcinoma may present a diagnostic dilemma. Carcinomas composed largely of neoplastic glandular tissue with a small, well-differentiated squamous cell component are probably best classified as adenocarcinomas.
- Squamous cell carcinoma: Composed largely of malignant squamous epithelium, however some tumors may have entrapped nests of nonneoplastic respiratory or submucosal gland epithelial cells.

(Boorman, Morgan, and Uraih 1990; Brown 1990; Dungworth, Ernst, et al. 1992; Dungworth, Hahn, Schuller, and Stinson 1992; Dungworth et al. 2001; Lee and Trochimowicz 1982; Reznik, 1994; Schüller, Gregg, and Reznik 1990; Woutersen et al. 1989)

Adenocarcinoma (Figure 45): Nasal Cavity, Nasopharynx, Paranasal Sinus

Pathogenesis/cell of origin: Malignant transformation of transitional, respiratory or glandular epithelium, or olfactory Bowman's glands.

Diagnostic features:

- Localized in the anterior nasal cavity (originating from subepithelial glands), in the posterior nasal cavity (often originating in the mucosa of the ethmoturbinates), or by malignant change occurring in an adenoma
- Solid, pseudoglandular, papillary or tubular formations
- Lumina may be filled with mucosubstances.
- Large cuboidal to columnar, or anaplastic cells
- Loss of polarity of the epithelium
- May show only penetration of the basement membrane and invasion of the surrounding bone, cribriform plate, or olfactory lobes of the brain
- Invasion of the cerebrum or metastases to regional lymph nodes or lung may occur.
- Areas of squamous differentiation may be present.

- Well-differentiated adenocarcinomas have prominent glandular or cystic structures lined by more regular secretory cells.
- Poorly differentiated adenocarcinomas have masses of anaplastic cells, inconspicuous glandular structures, a high degree of nuclear pleomorphism, and often abnormal mitotic figures.

Differential diagnoses:

- Adenoma: Absence of signs of malignancy such as penetration of the basement membrane, invasive growth, and metastases. Glandular structures, when present, usually round and lined by well-differentiated tall columnar cells, with basally located nuclei, which do not display marked cytologic atypia
- Adenosquamous carcinoma: The neoplastic squamous cell portions of the adenosquamous carcinoma show signs of malignancy such as frequent mitoses, cellular or nuclear atypia, or invasion into surrounding tissues, lymphatics, or vessels. Metaplastic squamous cell portions within an adenocarcinoma have more benign characteristics and are not as prominent
- Neuroepithelial carcinoma: Neurogenic structures, namely, plexiform intercellular fibrils ("neurofibrils") and/or rosettes, should be demonstrable

Comment: Location of the tumor usually determines the type of adenocarcinoma (transitional or respiratory epithelial). Transitional tumors are found mostly in the lateral meatus of the anterior (proximal) aspect of the nasal cavity. Occasionally, distinction between poorly differentiated adenocarcinoma and neuroepithelial carcinoma is possible only by electron microscopic analysis. When a neurogenic component is not demonstrable, a diagnosis of adenocarcinoma is preferable.

(Belinsky et al. 1987; Boorman, Morgan, and Uraih 1990; Brown 1990; Chen et al. 1995; Dungworth, Ernst, et al. 1992; Dungworth, Hahn, et al. 1992; Dungworth et al. 2001; Feron, Woutersen, and Spit 1986; Griciute et al. 1986; Lee and Trochimowicz 1982; Maronpot 1990; Pour and Götz 1983; Renne et al. 1986; Reznik, Schuller, and Stinson 1994; Reznik-Schüller 1983a, 1983b; Schüller, Gregg, and Reznik 1990; St. Clair and Morgan 1992; Stinson 1983; Stinson and Reznik 1985; Tamano et al. 1988; Yamamoto et al. 1989).

Carcinoma, Neuroepithelial (Figure 46): Nasal Cavity, Nasopharynx, Paranasal Sinus

Synonym(s) Neuroepithelioma, olfactory; neuroblastoma, olfactory; esthesioneuroblastoma; carcinoma, olfactory.

Pathogenesis/cell of origin: Malignant transformation of olfactory epithelium (sustentacular cells, basal cells, immature sensory cells, and possibly ductal cells of Bowman's glands).

Diagnostic features:

- Arises from the olfactory epithelium, which covers a small portion of the dorsocranial meatus and contiguous upper one-third of the nasal septum and the majority of the ethmoid turbinates in the posterior nasal cavity
- Frequently there is compartmentalization of sheets of neoplastic cells into lobules by fibrovascular septa
- Small round or columnar cells with poorly defined, pale-staining cytoplasm
- Round to oval, basally located nuclei that do not display marked cytologic atypia
- Distinct, sharply defined nuclear chromatin
- True (Flexner-Wintersteiner) rosettes (tumor cells surround an open central lumen bounded by distinct cell membranes, thus mimicking glandular structures) or pseudo (Homer-Wright) rosettes (tumor cells arranged in a circle around a small central lumen, which is filled with amorphous or tangled fibrillary material) may be present
- Plexiform intercellular fibrils
- Areas of anaplastic cells may be present.

Differential diagnosis:

- Adenocarcinoma: Absence of rosettes or plexiform intercellular fibrils. A useful distinguishing feature between true rosettes sometimes present in neuroepithelial carcinomas and the acinar spaces in adenocarcinomas is that in neuroepithelial carcinomas, the nuclei of cells forming the rosettes blend peripherally with the uniform population forming the rest of the tumor.

Comment: Often invades the ethmoid bone and brain. Frequency and morphology of rosette structures are highly variable. Whether tumors arising from olfactory epithelium are more appropriately classified under the term neuroepithelial carcinoma or olfactory neuroblastoma cannot be resolved on the basis of current information. The general term neuroepithelial carcinoma is used here because it allows for origin of neoplasms from sensory or sustentacular cells, whereas olfactory neuroblastoma implies origin only from neurogenic components. There is clearly a need to develop procedures to distinguish neuroepithelial tumors of neurogenic origin (neuroblastoma, esthesioneuroblastoma), from those of nonneurogenic cell types, especially sustentacular cells and their precursors. When neither rosettes (true or pseudo) nor plexiform intercellular fibrils are demonstrable, a diagnosis of adenocarcinoma is preferable. Ultrastructural analysis of a neuroepithelial carcinoma should reveal some clear characteristics of olfactory epithelium such as olfactory vesicles, cilia, and microtubules. The histogenic relationship of Bowman's glands to the other components of the nasal epithelium has not been clearly defined, but their origin is most likely nonneurogenic. Therefore, carcinomas originating from Bowman's glands should be classified with adenocarcinomas. Most cases of neuroepithelial carcinoma are negative for immunohistochemical reactions with antibodies against intermediate filaments.

(Boorman, Morgan, and Uraih 1990; Brown 1990; Dungworth, Ernst, et al. 1992; Dungworth, Hahn, et al. 1992;

Dungworth et al. 2001; Elkon 1983; Feron, Woutersen, and Spit 1986; Griciute et al. 1981; Rabstein and Peters 1973; Reznik, Schuller, and Stinson 1994; Rivenson et al. 1983; Schüller, Gregg, and Reznik 1990; Stinson 1983; Stinson and Reznik-Schueller 1985; Vollrath and Altmannsberger 1989; Vollrath and Altmannsberger 1989; Vollrath et al. 1986).

II. Larynx, Trachea, Bronchi, and Bronchioles

Although less complex than the epithelium lining nasal airways, the mucosal epithelium lining the laryngeal lumen consists of several distinct histologic subtypes with varying susceptibility to damage from inhaled toxicants (D. Lewis 1991; Renne and Miller 1996; Renne et al. 2003; Renne and Gideon 2006). The most frequent site of induced laryngeal lesions is the zone of transition between squamous and respiratory epithelium at the base of the epiglottis. This critical area for interpretation of toxic effects is rather small, and it is important to use histologic procedures that ensure it is consistently available for microscopic examination (D. Lewis 1991; Renne et al. 1992; Sagartz et al. 1992; Kittel et al. 2004). The epithelium lining the transition zone in rats is a mixture of slightly rounded, cuboidal, and ciliated columnar cells. In the mouse, this epithelium is nearly a pure population of columnar ciliated cells (Renne et al. 1992). Other sites frequently affected are the medial aspect of the arytenoid projections and the area anterior and lateral to the ventral pouch (D. Lewis 1991; R. R. Miller and Renne 1996).

The term bronchus refers to airways distal to the trachea, the walls of which contain cartilage, smooth musle, and submucosal glands (Mariassy 1992). The trachea and bronchi of rats are lined predominantly by pseudostratified, ciliated columnar (respiratory) epithelium, with lesser numbers of basal, serous, and mucous goblet cells (Mariassy 1992). Globule leucocytes are scattered among respiratory epithelium lining the larynx and trachea of rats. Jecker et al. (1996) described the distribution of immunocompetent cells in various areas of the rat larynx. Widdicombe et al. (2001) described interspecies differences in the numbers and distribution of submucosal glands of the larynx and trachea of rodents and rabbits. The height of the pseudostratified tracheal epithelium decreases caudally to simple epithelium in the bronchi. The walls of only the extrapulmonary mainstem bronchi of rats contain supporting cartilage. Nodules of bronchus-associated lymphoid tissue (BALT) are closely associated with the proximal intrapulmonary bronchi.

The anatomical structure of the tracheobronchial tree determines, in part, the dose of an inhaled irritant delivered to a specific airway. The epithelium lining the medial surfaces at the tip of the tracheal bifurcation (carina) is a common site for lesions induced by inhaled irritants, presumably because this area receives a higher dose from direct aerosol impaction (Gopinath, Prentice, and Lewis 1987). Therefore, in inhalation studies, the carina should be included in airway sections taken for histopathological evaluation.

Systematic sampling and careful fixation by airway perfusion are prerequisites to identifying lung lesions. In the rat and mouse lung, the trachea bifurcates at the carina and gives rise to the two extrapulmonary bronchi that enter the left and right lung lobes and ramify as twelve to twenty monopodal branching airway segments of decreasing caliber from the trachea to the terminal bronchiole, depending upon the lobe (Yeh and Harkema 1993). The right primary bronchus branches to a cranial lobar bronchus, followed by a middle and accessory lobar bronchus, and terminates in a caudal lobe bronchus. The left primary bronchus serves the single large left lobe (Hebel and Stromberg 1986). Intralobular septa are absent, as are lobules.

For purposes of organization in this document, lesions affecting proximal bronchioles are grouped with larynx, trachea, and bronchi and lesions affecting terminal bronchioles are grouped with alveoli. The bronchiolar mucosa is lined with a mixture of simple columnar ciliated and Clara cells, with numbers of ciliated epithelium increasing distally (Boorman and Eustis 1990; Plopper and Hyde 1992). Submucosal glands are absent.

A. Epithelial Changes: Larynx, Trachea, Bronchi, Bronchioles

Degeneration (Figures 47 and 48): Larynx, Trachea, Bronchi, Bronchioles

Pathogenesis/cell of origin: Squamous, transitional, respiratory and glandular epithelium.

Diagnostic features:

- Loss of cilia (respiratory epithelium only)
- Epithelial blebbing or cytoplasmic vacuolation
- Rounding up of normally cuboidal/columnar epithelium
- Pyknosis of nuclei
- Dilatation (ectasia) of submucosal glands
- Accumulation of secretory material in glands as sequel to blockage of duct resulting from squamous metaplasia

Differential diagnoses:

- Postmortem autolysis: Uniform dissolution of entire tissue section with no change in organization or depth of cell layers
- Normal nonciliated epithelium for that location
- Artifactual damage from microtome or insertion of cannula
- Spontaneous accumulation of secretory material

Comment: Degeneration of epithelium lining the laryngeal lumen is a frequently observed effect of inhaled materials of low toxicity or of lower concentrations of potent toxicants (R. A. Miller and Renne 1996). Subtle changes, including loss of cilia and rounding of normally cuboidal or columnar epithelial cells, are most often observed at the base of the epiglottis. Care must be taken to distinguish these changes from cuboidal or rounded, nonciliated epithelial cells normally

present in the transition zone of the epiglottis (D. Lewis 1991; R. A. Miller and Renne 1996). Degeneration of tracheal epithelium occurs less frequently. Decreases in number or complete loss of globule leucocytes normally present in laryngeal and tracheal epithelium of rats is a frequently observed response to inhaled chemicals (D. Lewis 1991). Accumulation of secretory material in submucosal gland ducts may also occur spontaneously.

Necrosis (Figures 49 and 50): Larynx, Trachea, Bronchi, Bronchioles

Pathogenesis/cell of origin: Squamous, transitional, respiratory and glandular epithelium.

Diagnostic features:

- Pyknosis, karyorrhexis of nuclei
- Acute inflammatory response
- Sloughing of affected epithelium

Differential diagnoses:

- Postmortem autolysis: Uniform dissolution of entire tissue section with no change in organization or depth of cell layers
- Artifactual damage

Comment: Although necrosis is seen most frequently in the transition zone at the base of the epiglottis, inhalation of strong irritants can induce severe lesions in the entire lumen, with ulceration and severe inflammation (R. A. Miller and Renne 1996). Severe necrosis of the upper respiratory tract and bronchial mucosa has been induced by inhalation exposure to strong irritants such as methyl isocyanate (Boorman and Eustis 1990).

Erosion/Ulceration (Figures 51 and 52): Larynx, Trachea, Bronchi, Bronchioles

Pathogenesis/cell of origin: Squamous, transitional, respiratory and glandular epithelium.

Diagnostic features:

- Loss of mucosal epithelium only (erosion)
- Complete penetration of underlying basement membrane (ulceration)
- Associated inflammation, usually suppurative or serofibrinous

Differential diagnoses:

- Developmental defect: Loss of epithelium but no underlying submucosa, no evidence of necrosis or inflammation

Comment: Erosion and ulceration are most likely to occur in the mucosal epithelium lining the base of the epiglottis. A serofibrinous or suppurative exudate is usually visible covering areas of ulcerated epithelium. Care must be taken to distinguish erosion or ulceration from artifactual loss of epithelium related to experimental procedures or resulting from postmortem autolysis. Accidental introduction of a gavage tube into the airway lumen or poor intratracheal instillation technique can cause laryngeal or tracheal injury and consequently, stertor and serous or hemorrhagic nasal discharge. Severe laceration of laryngeal or tracheal tissues can result in subcutaneous emphysema resulting from air escaping into adjacent tissues. Microscopically, submucosal edema, congestion, or hemorrhage may be present in association with ulceration of the laryngeal or tracheal mucosae. Ulceration of mucosal epithelium may result in necrosis and mineralization of underlying cartilage. The "U-shaped" cartilage at the entrance to the ventral pouch is particularly susceptible to necrosis related to ulceration, and subtle lesions may persist in this cartilage after the overlying epithelium has returned to normal (D. Lewis 1991; Hardy et al. 1997). Squamous epithelial metaplasia may occur following repeated loss of the respiratory epithelium.

Ectasia in Submucosal Glands (Figure 53): Larynx, Trachea, Bronchi

Pathogenesis/cell of origin: Submucosal glands and associated ductal epithelium.

Diagnostic features:

- Abnormal dilatation of glands following blockage of excretory ducts secondary to hyperplasia or squamous metaplasia of overlying mucosal epithelium
- Inflammation secondary to accumulated secretions from blocked submucosal glands of the larynx, pharynx, and trachea with degeneration of associated cartilage
- Dilatation of submucosal glands of the larynx or trachea may also occur spontaneously

Differential diagnosis:

- Primary inflammation in glands: No evidence of blocked ducts

Comment: Abnormal dilatation of glands follows blockage of excretory ducts resulting from hyperplasia or squamous metaplasia of the overlying mucosal epithelium or accumulation of exudate within the ducts (D. Lewis 1991). Rupture of the ectatic glands with release of accumulated necrotic debris induces a granulomatous inflammatory response that may progress to severe polypoid lesions that partially occlude the laryngeal lumen (Bucher et al. 1990; Schwartz et al. 1994). Submucosal gland granulomas with degeneration of associated cartilages have been reported in the larynx, pharynx, and trachea (Germann, Ockert, and Heinrichs 1998). These lesions were considered related to injury during oral gavage, and they were more frequently observed in Fischer 344 rats than in other strains. Dilatation of submucosal glands of the larynx or trachea may also occur spontaneously (Greaves and Faccini 1984).

Regeneration: Larynx, Trachea, Bronchi, Bronchioles
Pathogenesis/cell of origin: Squamous, transitional, respiratory, and glandular epithelium.
Diagnostic features:

- Normal appearing epithelial cells with basophilic cytoplasm
- Increased nuclear:cytoplasmic ratio
- Epithelial architecture may remain irregular
- Adjacent to or within areas of epithelial degeneration, necrosis, hyperplasia, or metaplasia

Differential diagnoses:

- Hyperplasia: Epithelium is thickened owing to increased numbers of cells, resulting in undulating, rugose epithelial surface and irregular arrangement of cell layers (see proliferative lesion section of this document)
- Neoplasia: Expansile nodule usually protruding into the lumen, with cellular atypia and compression of adjacent structures (see proliferative lesion section of this document)

Comment: Sequelae to injury of laryngeal, tracheal, or bronchial epithelium are similar to those described above for nasal epithelium. Epithelial degeneration and regeneration may be present together in laryngeal or tracheal tissues repeatedly exposed to injurious chemicals and may result in a disorganized histologic appearance. Sequelae range from rapid regeneration of epithelium identical to the original epithelium to squamous metaplasia. Repeated loss of transitional or respiratory epithelium leads to squamous metaplasia, usually accompanied by hyperplasia and, frequently, keratization of metaplastic epithelium. Squamous metaplasia is described in detail and illustrated in the proliferative lesions section of this guide. It is important to attempt to distinguish well-established squamous metaplasia following repeated injury to respiratory epithelium from the flattened reparatory squamous-like epithelium that temporarily covers areas of respiratory epithelium recently denuded during epithelial repair (K. Keenan 1987). Cytokeratin expression patterns in the epithelia lining the larynx, trachea, and bronchi that could aid in making this distinction have been reported (Schlage, Bulles, Friedrichs, Kuhn, and Teredesai 1998; Schlage, Bulles, Friedrichs, Kuhn, Teredesai, and Terpstra 1998).

B. Inflammation: Larynx, Trachea, Bronchi, Bronchioles

Synonyms: laryngitis, tracheitis, bronchitis.

Inflammation, Acute (Figures 50–52): Larynx, Trachea, Bronchi, Bronchioles
Pathogenesis/cell of origin: Squamous, transitional, respiratory, or glandular epithelium and associated tissues and cavities.
Diagnostic features:

- Vascular congestion
- Edema
- Accumulation of serous, fibrinous, or suppurative inflammatory exudates

Differential diagnoses:

- Chronic active inflammation: Mixture of granulocytic, lymphocytic, and histiocytic inflammatory cells and fibrosis
- Agonal hemorrhage and congestion: No or very few inflammatory cells
- Postmortem autolysis: Uniform dissolution of entire tissue section with no change in organization or depth of cell layers

Comment: The base of the epiglottis and ventral pouch are frequently the sites first and most severely affected by acute inflammation from inhaled irritants. Migration of neutrophils into airways produces a suppurative exudate. Eosinophils in exudate or mucosal infiltrate may indicate an immunologic element to the inflammation (Ibanes et al. 1996). A variable inflammatory response characteristically accompanies degenerative or proliferative lesions induced in the rodent larynx, trachea, or bronchi. The site of inflammation and type and severity of inflammatory response depends on the physical characteristics of the inducing agent and the duration of exposure. A mild suppurative inflammatory infiltrate is typically present in areas of laryngeal epithelial degeneration, becoming more severe and often accompanied by bacterial colonization when ulceration of mucosal epithelium occurs (D. Lewis 1991). Infectious agents affecting the nasal cavity may also cause inflammatory lesions in the larynx, trachea, and bronchi and may create problems in differential diagnosis of subtle lesions at these sites. Inhalation of highly irritant compounds can induce fatal inflammatory lesions in the laryngeal mucosa. Severe laryngeal edema and fibrin or mucus accumulation caused death from airway occlusion in rats inhaling high concentrations of red phosphorus smoke or combustion products of sodium and lithium (Burton et al. 1982; R. A. Miller and Renne 1996; Rebar, Greenspan, and Allen 1986).

Inflammation, Granulomatous: Larynx, Trachea, Bronchi, Bronchioles
Pathogenesis/cell of origin: Squamous, transitional, or respiratory epithelium and associated tissues and cavities.
Diagnostic features:

- Cellular infiltrate is predominantly plump macrophages (epithelioid cells), which may form interlacing bundles, and is accompanied by lymphocytes,

plasma cells, and fibrosis, depending on duration and etiologic agent.
- Infiltrating macrophages may form multinucleated giant cells.
- Etiologic agent may be visible, for example, fungi, mycobacteria, or foreign bodies.
- Granulocytes may be present in the affected area, in which case the process may be described as pyogranulomatous inflammation.
- Hyperplasia or metaplasia of affected epithelium may be present.

Differential diagnoses:

- Other types of inflammation (see below)
- Early connective tissue or hematopoietic neoplasia

Comment: Granulomatous inflammation suggests an etiologic agent which is resistant to dissolution or is immunogenic, for example, fungi, mycobacteria, or foreign body (Kumar, Abbas, and Fausto 2004). An example is the inflammatory response to exudate trapped in the lumens of ectatic submucosal glands.

Inflammation, Chronic (Figures 54 and 55): Larynx, Trachea, Bronchi, Bronchioles
Pathogenesis/cell of origin: Squamous, transitional, respiratory, or glandular epithelium and associated tissues and cavities.

Diagnostic features:

- Infiltrates of lymphocytes, macrophages, and fibroblasts into the lamina propria
- Hyperplasia of mucosal-associated lymphoid tissues (MALT)
- Hyperplasia or metaplasia of overlying epithelium

Differential diagnoses:

- Resolving acute inflammation: Neutrophils included in the inflammatory cell infiltrate
- Early connective tissue or hematopoietic neoplasia

Comment: Subcategories or variants of chronic inflammation (chronic active or granulomatous inflammation) of the larynx, trachea, or bronchi have features similar to those described above for the nasal cavity; infiltration of predominantly mononuclear inflammatory cells, hyperplasia of associated lymphoid tissue, and metaplasia or hyperplasia of affected epithelium. Epithelial metaplasia and hyperplasia are described and illustrated in the proliferative lesions section of this document and elsewhere (Dungworth, Ernst, et al. 1992; Schwartz et al. 1994). Mineralized debris, hairs, or inhaled food material accompanied by a mixed inflammatory cell exudate and epithelial changes (e.g., squamous metaplasia) are frequently observed in the lumen of the ventral pouch of the rat larynx, occurring more frequently in aged animals (D. Lewis 1991; R. A. Miller and Renne 1996). Similar cystic lesions may occur in submucosal glands of the trachea secondary to inflammation or squamous metaplasia of lumenal epithelium.

Bronchiectasis (Figure 56): Larynx, Trachea, Bronchi, Bronchioles
Pathogenesis/cell of origin: Bronchial epithelium, lumen, and adjacent tissue

Diagnostic features:

- Dilatation of bronchial lumen with mucopurulent exudate filling the lumen
- Fibrosis around affected bronchi

Differential diagnoses:

- Congenital abnormalities: Lack or decrease of inflammatory infiltrate or fibrosis
- Inhaled foreign bodies: Visible in macrophages or extracellularly

Comment: Bronchiectasis is a common feature of bronchopneumonia caused by *Mycoplasma pulmonis*, now a very rarely observed disease of laboratory rats. A mucopurulent exudate partly or completely fills the mainstem bronchi and may extend to adjacent bronchioles. Severe dilatation of bronchi occurs to compensate for decreased intrapulmonary space created by atelectasis distal to the blocked airways.

C. Vascular Changes: Larynx, Trachea, Bronchi, Bronchioles

Changes in the vasculature of the larynx, trachea, and bronchi are similar to those described above for the nasal cavity or below for bronchioles and alveoli.

D. Nonneoplastic Proliferative Lesions: Larynx, Trachea, Bronchi, Bronchioles

Epithelial Alteration (Figure 57): Larynx
Pathogenesis/cell of origin: Respiratory and/or cuboidal/transitional epithelium.

Diagnostic features:

- Loss of cilia (respiratory epithelium)
- Focal or diffuse flattening of epithelial cells
- Slight increase in cell layers (three to four)

Differential diagnoses:

- Squamous cell metaplasia: Characterized by more cell layers and replacement of respiratory epithelium by squamous epithelial cells, which may exhibit slight nuclear polymorphism and cellular atypia; surface cells may be highly keratinized or contain only

keratohyalin granules. Desquamation of surface cells may occur
- Re-epithelialization following ulceration or erosion of surface epithelium (see Erosion/Ulceration, above)

Comment: The sites most susceptible to development of this lesion are the base of the epiglottis and the area around the ventral pouch (Kaufmann et al. 2009). Use of this nonspecific diagnostic term requires further characterization of the lesion in the narrative of any pathology report.

Metaplasia, Squamous Cell (Figures 58 and 59): Larynx, Trachea, Bronchi, Bronchioles

Pathogenesis/cell of origin: Metaplasia of respiratory epithelium or submucosal glands.

Diagnostic features:

- Focal or diffuse replacement of respiratory or ductal epithelium by squamous epithelium
- Surface cells may be nonkeratinized, keratinized, and desquamating, and may contain keratohyaline granules.
- Cells may show distinct intercellular bridges.
- Nuclei mostly round to oval with a distinct nucleolus, but in some areas may be flat or deeply invaginated.
- Slight nuclear polymorphism and cellular atypia may be present.
- Frequently associated with pseudostratified/stratified hyperplasia of squamous epithelium.

Differential diagnoses:

- Papilloma: Projection above the surface of the respiratory epithelium or extension into the lumen of the ducts of the submucosal glands. Presence of delicate cores of vascular connective tissue stroma covered by marked proliferative thickening of the epithelium
- Squamous cell carcinoma: Characterized by more pronounced cellular atypia or disorganization or invasion of basement membrane or adjacent tissue structures
- Epithelial alteration (applies principally to laryngeal epithelium): Slight modification of epithelial cells, namely, loss of cilia, flattening and horizontal orientation of epithelial cells, three to four cell layers
- Re-epithelialization following ulceration or erosion of surface epithelium (see Erosion/Ulceration, above)

Comment: The laryngeal sites most susceptible to squamous cell metaplasia in mice and rats are at the base of the epiglottis and in the laryngeal pouch, where the epithelium overlies a small cluster of submucosal glands. Accurate planes of section and precise anatomic localization are necessary for proper evaluation of squamous metaplasia at these sites. Squamous metaplasia may occur in association with acute and/or chronic inflammation or in the process of regeneration. It is important to distinguish re-epithelialization following ulceration or erosion of surface epithelium from true squamous metaplasia (see Erosion/Ulceration, above). Since normal stratified squamous epithelium contains more cell layers than normal respiratory epithelium, a diagnosis of squamous metaplasia infers an increase in numbers of cell layers. However, a separate diagnosis of hyperplasia of metaplastic squamous epithelium (see Squamous Cell Hyperplasia, below) may be used when there is a need to indicate the severity of the hyperplasia. Squamous metaplasia with normal differentiation pattern is reversible under some experimental circumstances, depending on the nature of inhaled irritant and the duration of exposure, but in other situations it may give rise to squamous papillomas or squamous cell carcinomas.

(Boorman, Morgan, and Uraih 1990; Dickhaus et al. 1977; Dickhaus et al. 1978; Dungworth, Ernst, et al. 1992; Dungworth et al. 2001; Faccini, Abbott, and Paulus 1990; Gopinath, Prentice, and Lewis 1987; Green et al. 1980; Karube et al. 1989; Kerns et al. 1983; Luts et al. 1991; Maekawa and Odashima 1975; Maronpot 1990; Maronpot et al. 1986; Pack, Al-Ugaily, and Morris 1981; Pour et al. 1976; Rehm and Kelloff 1991; Rehm, Ward, and Sass 1994; Reznik, Schuller, and Stinson 1980; Reznik 1983; Takahashi, Iwasaki, and Ide 1985; Yamamoto et al. 1989).

Hyperplasia, Squamous Cell (Figure 60): Larynx, Trachea, Bronchi, Bronchioles

Pathogenesis/cell of origin: Proliferation of normal squamous epithelium of the larynx or of metaplastic squamous epithelium in the larynx, trachea, or bronchi.

Diagnostic features:

- Proliferation and thickening of the squamous epithelium normally lining portions of the larynx (see Metaplasia, Squamous, above) or of metaplastic squamous epithelium in the trachea or bronchi
- Focal increase in the number of cell layers normally present
- Normal differentiation pattern
- Occasional mitoses are observed.
- The hyperplastic squamous cells have larger nuclei, more prominent nucleoli and more abundant cytoplasm.

Differential diagnoses:

- Papilloma, squamous cell: Squamous cell papilloma is characterized by exophytic growth of squamous cells, which may be mixed with cuboidal or mucus-producing cells, resting on a vascularized stalk of connective tissue.
- Squamous or adenosquamous carcinoma: Epithelial hyperplasia with cellular atypia might progress to squamous or adenosquamous carcinoma.

Diagnosis of malignant tumor is based on one or more of overall size, loss of polarity of epithelium, high degree of atypia or mitotic index, or invasive behavior.

Comment: Since the thickness of the normal laryngeal squamous epithelium varies slightly depending upon the location, precise sectioning and careful comparison to controls is important. Severe squamous metaplasia, hyperplasia, and inflammation accompanied by proliferation of a highly vascular connective tissue stalk may occur as a sequel to blockage of laryngeal submucosal gland ducts by metaplastic duct epithelium (see Ectasia, Submucosal Glands, above). These lesions, classified as inflammatory polyps, may progress to partially block the laryngeal lumen (Bucher et al. 1990).

(Boorman, Morgan, and Uraih 1990; Dickhaus et al. 1977; Dickhaus et al. 1978; Dungworth, Ernst, et al. 1992; Dungworth et al. 2001; Faccini, Abbott, and Paulus 1990; Gopinath, Prentice, and Lewis 1987; Green et al. 1980; Karube et al. 1989; Luts et al. 1991; Maekawa and Odashima 1975; Maronpot 1990; Maronpot et al. 1986; Pack, Al-Ugaily, and Morris 1981; Pour et al. 1976; Rehm and Kelloff 1991; Rehm, Ward, and Sass 1994; Reznik 1983; Takahashi Iwasaki, and Ide 1985; Yamamoto et al. 1989)

Hyperplasia, Respiratory Epithelium (Figure 61): Larynx, Trachea, Bronchi, Bronchioles

Pathogenesis/cell of origin: Proliferation of respiratory or glandular epithelium.

Diagnostic features:

- Increased layers of surface respiratory epithelial cells, usually lacking cilia in the larynx
- May form luminal protrusions in trachea or bronchi
- Papillary hyperplasia present when small fronds of epithelium with simple connective tissue core project into the airway lumen
- Mild nuclear atypia and pleomorphism may be detected.

Differential diagnoses:

- Papilloma: Papillary projection above the surface of the respiratory epithelium or endophytic (inverted) growth. Presence of cores of delicate vascular mesenchymal stroma covered by marked proliferative thickening of the epithelium
- Adenocarcinoma: Increased cellular atypia, or destruction of the basement membrane or other signs of malignancy such as invasion or destruction of adjacent tissues
- Metaplasia, squamous cell: Replacement of respiratory epithelium by mature squamous epithelium that frequently has superficial keratinization

Comment: In transgenic mice, papillary respiratory epithelial hyperplasia is frequently associated with papillomas or can be induced by multiple N-nitrosodiethylamine treatments, or intratracheal applications of carcinogens. Additional subclassification of hyperplasia may be appropriate, especially in experimental studies using agents that target specific cell types (e.g., Clara cells), but are not essential for routine toxicologic pathology.

(Boorman, Morgan, and Uraih 1990; Dungworth, Ernst, et al. 1992; Dungworth et al. 2001; Gopinath, Prentice, and Lewis 1987; Karube et al. 1989; Kaufmann et al. 2009; Kerns et al. 1983; D. Lewis 1991; Luts et al. 1991; Maronpot et al. 1986; Pack, Al-Ugaily, and Morris 1981; Rehm and Kelloff 1991; Takahashi, Iwasaki, and Ide 1985)

Hyperplasia, Mucous Cell (Figure 62): Larynx, Trachea, Bronchi, Bronchioles

Synonym(s): metaplasia, mucous cell; metaplasia/hyperplasia, goblet cell; metaplasia, mucous cell.

Pathogenesis/cell of origin: Proliferation of mucous cells within the respiratory epithelium.

Diagnostic features:

- Replacement of respiratory epithelium by mucous cells either as a single layer or as a pseudostratified layer

Differential diagnoses:

- Papilloma: Characterized by expansile/compressive growth of papillary structures
- Adenocarcinoma: Increased cellular atypia, or destruction of the basement membrane or other signs of malignancy such as invasion or destruction of adjacent tissues
- Metaplasia, squamous cell: Replacement of respiratory epithelium with regular squamous epithelium that frequently has superficial keratinization

Comment: Epithelial hyperplasia of mucous cells may occur in association with subacute and chronic inflammation caused by infectious agents or result from exposure to local carcinogenic or noncarcinogenic irritants. Secretory cells of the trachea, bronchi, and bronchioles may also contain eosinophilic globules (eosinophilic intracytoplasmic proteinaceous accumulations), a feature similar to that observed in the epithelium of the nasal cavity. These eosinophilic globules are considered dilated endoplasmic reticulum containing proteinaceous material and not a dysplastic alteration. They may occur in response to tissue irritation and often increase in incidence with age.

(Boorman, Morgan, and Uraih 1990; Dungworth, Ernst, et al. 1992; Dungworth et al. 2001; Gopinath, Prentice, and Lewis 1987; Karube et al. 1989; Kerns et al. 1983; D. Lewis 1991; Luts et al. 1991; Maronpot et al. 1986; Pack, Al-Ugaily, and Morris 1981; Rehm and Kelloff 1991; Takahashi, Iwasaki, and Ide 1985)

Hyperplasia, Neuroendocrine Cell (Figure 63): Larynx, Trachea, Bronchi, Bronchioles

Synonym(s): Hyperplasia, pulmonary neuroendocrine cell (PNEC).

Pathogenesis/cell of origin: Proliferation of neuroendocrine cells of bronchioles.

Diagnostic features:

- Clusters of uniform small cells that protrude into the lumen of the larynx, trachea, bronchi, or bronchioles. Cells have scant cytoplasm and nuclei with stippled chromatin.
- The lesion consists of more than forty neuroendocrine cells.
- Definitive identification of neuroendocrine cells requires immunostaining for specific markers. For the rat, protein gene product 9.5 (PGP), and calcitonin are suitable markers. For the mouse, the best markers appear to be protein gene product 9.5 (PGP), calcitonin, calcitonin gene–related peptide (CGRP), and helodermin.

Differential diagnoses:

- Papilloma: Characterized by expansile/compressive growth of papillary structures
- Adenocarcinoma: Increased cellular atypia, or destruction of the basement membrane or other signs of malignancy such as invasion or destruction of adjacent tissues
- Squamous metaplasia: Replacement of respiratory epithelium by mature squamous epithelium that is frequently keratinized

Comment: Bronchi and bronchioles are both included as anatomic locations in the description above because of differences in the basis for nomenclature of conducting airways in rodent lungs, namely, whether to classify according to type of epithelium or to presence/absence of cartilage. Small clusters of neuroendocrine cells that protrude slightly into the airway lumen are normal histologic features, are also referred to as neuroepithelial bodies (NEB), and must be distinguished from the larger hyperplastic lesions. In neuroendocrine cells of the larynx and trachea, serotonin could not be demonstrated, whereas in the lung, serotonin-containing cells were seen.

(Adriaensen et al. 2001; Boorman, Morgan, and Uraih 1990; Dungworth, Ernst, et al. 1992; Dungworth et al. 2001; Elizegi et al. 2001; Gopinath, Prentice, and Lewis 1987; Haworth et al. 2007; Karube et al. 1989; Kasacka and Sawicki 2004; Kerns et al. 1983; Larson et al. 2004; Lauweryns and Van Ranst 1988; Lauweryns et al. 1987; D. Lewis 1991; Luts et al. 1991; Maronpot et al. 1986; McBride et al. 1990; Montuenga et al. 1992; Pack, Al-Ugaily, and Morris 1981; Rehm and Kelloff 1991; Shimosegawa and Said 1991; Takahashi, Iwasaki, and Ide 1985; Van Lommel 2001; Van Lommel et al. 1999)

Hyperplasia with Cellular Atypia (Dysplasia): Larynx, Trachea, Bronchi, Bronchioles

Pathogenesis/cell of origin: Proliferation of respiratory epithelium, or submucosal glands.

Diagnostic features:

- Variation in cellular/nuclear size and shape of lining cells
- Increased nuclear basophilia
- Increased nuclear invaginations
- Bi- or multinucleated cells

Differential diagnoses:

- Papilloma: Characterized by expansile/compressive growth of papillary structures
- Adenocarcinoma: Increased cellular atypia, or destruction of the basement membrane or other signs of malignancy such as invasion or destruction of adjacent tissues
- Squamous metaplasia: Replacement of respiratory epithelium by mature squamous epithelium that frequently has superficial keratinization

Comment: Bronchi and bronchioles are both included as anatomic locations in the description above because of differences in the basis for nomenclature of conducting airways in rodent lungs, namely, whether to classify according to type of epithelium or to presence/absence of cartilage. Epithelial hyperplasia with cellular atypia is observed primarily following the systemic application of nitrosamines or after intratracheal instillation carcinogens. Cellular atypia is also a common feature of neoplasia and may be present without hyperplasia.

(Boorman, Morgan, and Uraih 1990; Dickhaus et al. 1977; Dickhaus et al. 1978; Dungworth, Ernst, et al. 1992; Dungworth et al. 2001; Faccini, Abbott, and Paulus 1990; Gopinath, Prentice, and Lewis 1987; Green et al. 1980; Karube et al. 1989; Kerns et al. 1983; D. Lewis 1991; Luts et al. 1991; Maekawa and Odashima 1975; Maronpot 1990; Maronpot et al. 1986; Pack, Al-Ugaily, and Morris 1981; Pour et al. 1976; Rehm and Kelloff 1991; Rehm Ward, and Sass 1994; Reznik 1983; Takahashi, Iwasaki, and Ide 1985; Yamamoto et al. 1989)

E. Neoplastic Proliferative Lesions: Larynx, Trachea, Bronchi, Bronchioles

Papilloma (Figures 64 and 65): Larynx, Trachea, Bronchi, Bronchioles

Pathogenesis/cell of origin: Neoplastic proliferation of respiratory or squamous epithelium.

Diagnostic features:

- Airway is expanded or distorted by growth of branching papillary structures with central connective tissue stalk and lined by cuboidal/respiratory epithelial cells

- Because of the plane of sectioning, may appear to arise from airway epithelium
- In some experimental studies, is frequently associated with papillary hyperplasia
- If papilloma originates in terminal bronchioles, there may be growth by expansion into alveolar parenchyma
- Intact basement membrane
- No clear evidence of invasion of adjacent structures
- The branching connective tissue stalk is usually lined by various proportions of cuboidal or columnar respiratory epithelium, but may occasionally be lined by squamous cells
- Mitotic figures rare and limited to the basal layers of the epithelium

Differential diagnoses:

- Hyperplasia, respiratory epithelium: Absence of central connective tissue stalk or absence of luminal expansion/distortion by branching growth
- Metaplasia, squamous cell: Absence of central connective tissue stalk or absence of luminal expansion/distortion by branching growth. See description of inflammatory polyps above under Metaplasia, Squamous.
- Adenocarcinoma: Characterized by increased cellular pleomorphism or invasion of adjacent pulmonary structures
- Squamous cell carcinoma: Destruction of the basement membrane, or cellular atypia, disorientation, more frequent mitoses, or presence of other signs of malignancy such as invasive growth or metastases
- Adenoma or carcinoma, bronchiolo-alveolar: These neoplasms arise in alveolar parenchyma. The adenoma may extend with papillary growth from an alveolar duct into a terminal bronchiole. The carcinoma is more invasive or destructive.

Comment: Severe squamous metaplasia, hyperplasia, and inflammation accompanied by proliferation of a highly vascular connective tissue stalk may occur as a sequel to blockage of laryngeal submucosal gland ducts by metaplastic duct epithelium (see Ectasia, Submucosal Glands, above). These lesions, classified as nonneoplastic inflammatory polyps, may progress to partially block the laryngeal lumen (Bucher et al. 1990). Distinguishing between a papilloma originating in a small bronchiole and a papillary bronchiolo-alveolar adenoma may not be possible (or necessary). Unless there is clear evidence that the tumor arose from airway epithelium, it should be classified in the bronchiolo-alveolar category. Diagnosis of bronchiolar papilloma may be appropriate in some experimental studies for papillomas that arise from the epithelium of small airways, and that should be distinguished from the usual types of bronchiolo-alveolar tumors that occur spontaneously in the lungs of mice. Bronchial papillomas lined by uniform cuboidal to columnar cells, and whose crowded papillary structures mimic a glandular pattern, are sometimes referred to as bronchial adenomas.

(Boorman and Eustis 1990; Boorman, Morgan, and Uraih 1990; Dickhaus et al. 1977; Dickhaus et al. 1978; Dungworth, Ernst, et al. 1992; Dungworth, Hahn, et al. 1992; Dungworth et al. 2001; Ito et al. 1989; Faccini, Abbott, and Paulus 1990; Gopinath, Prentice, and Lewis 1987; Green et al. 1980; Karube et al. 1989; Luts et al. 1991; Maekawa and Odashima 1975; Maronpot 1990; Maronpot et al. 1986; Maronpot et al. 1991; Mohr et al. 1990; Pack, Al-Ugaily, and Morris 1981; Pour et al. 1976; Rehm and Kelloff 1991; Rehm, Ward, and Sass 1994; Reznik 1983; Takahashi, Iwasaki, and Ide 1985; Schüller 1987; Yamamoto et al. 1989)

Tumor, Neuroendocrine Cell, Benign: Larynx, Trachea, Bronchi, Bronchioles

Synonym(s): Papilloma, neuroendocrine; Papilloma, pulmonary neuroendocrine cells (PNEC).

Pathogenesis/cell of origin: Neoplastic proliferation of the dispersed neuroendocrine cells in the tracheal, bronchial or bronchiolar epithelium.

Diagnostic features:

- Nodular thickening of tracheal or bronchial wall with narrowing of lumen
- In larger lesions, tumor cells separated into lobules and cords by sparse fibrovascular stroma
- Polygonal cells with distinct cell borders and abundant pale, finely granular cytoplasm
- Mitotic figures rare
- No invasion of airway wall

Special techniques for diagnosis:

- Definitive identification of neuroendocrine cells requires immunostaining for specific markers or Grimelius silver stain:
  - Rat and mouse: protein PGP and calcitonin are suitable markers.
  - Mouse: CGRP and helodermin are additional markers.
  - Grimelius silver stain argyrophilic neurosecretory granules in the cytoplasm.

Differential diagnoses:

- Hyperplasia, neuroendocrine cell: Clusters of uniform small cells which protrude into the lumen of bronchioles. Cells have scant cytoplasm and nuclei with stippled chromatin. The lesion consists of many neuroendocrine cells, but lacks fibrovascular stroma
- Tumor, neuroendocrine cell, malignant: Invasion of airway wall.
- Papilloma: Histochemical, immunohistochemical, and ultrastructural methods necessary to make