definitive diagnosis and to distinguish this tumor from papilloma.

Comment: Neuroendorine papillomas in the bronchi of rats are rare, and tumors with positive immunoreactivity for calcitonin have been reported. These tumors were induced by exposure to urban ambient air and were characterized by papillary proliferation of nonciliated epithelial cells with finely granulated, round nuclei and lightly eosinophilic cytoplasm and fibrous stroma. Papillary hyperplasias of neuroendocrine cells are described in the area of bronchi to bronchiolo-alveolar junction. Although neuroendocrine cell hyperplasias in mice are described above, there are no reports of neuroendocrine tumors in mice.

(Elizegi et al. 2001; Haworth et al. 2007; Ito et al. 1989; Kasacka and Sawicki 2004; Larson et al. 2004; Lauweryns and Van Ranst 1988; McBride et al. 1990; Montuenga et al. 1992; Shimosegawa and Said 1991; Van Lommel 2001; Van Lommel et al. 1999)

Carcinoma, Squamous Cell (Figures 66 and 67): Larynx, Trachea, Bronchi, Bronchioles

Synonym(s): Carcinoma, epidermoid.

Pathogenesis/cell of origin: Malignant transformation of respiratory epithelium (or squamous epithelium of larynx) that has undergone squamous metaplasia and progressed to neoplasia.

Diagnostic features:

- Growth pattern in cell clusters or irregular structures with central keratinization (keratin pearls), or without overt keratinization but forming distinct intercellular bridges. Cellular debris and necrosis may be common, as well as the presence of inflammatory cells in particular neutrophils.
- Shape, size, and orientation of cells: Irregular, large, and polygonal or flattened and stratified cells
- Cytoplasm may frequently be eosinophilic and granular to hyalinized because of the high keratin content
- Cytologic features common to squamous cell carcinoma, that is, dysplasia to anaplasia
- Cells may frequently be quite pleomorphic, including the formation of very large cells ("giant cells") 60 μm or more at the largest diameter
- Mitoses frequent in some areas
- Penetration of the basement membrane and invasion of adjacent tissues
- Scirrhous response may occur in association with invasion.

Differential diagnoses:

- Metaplasia, squamous cells: Shows no or minimal exophytic growth. Does not form papillary projections. Regular, orderly maturation with or without keratinization and little or no dysplasia or atypia. No vascularized stalk of connective tissue stroma.
- Papilloma: Intact basement membrane. Absence of features of malignancy such as frequent mitoses, cellular atypia, invasion into surrounding tissues, lymphatics, vessels, or bronchi. Exophytic growth.
- Adenocarcinoma: Composed of papillary strands lined by cuboidal or pleomorphic cells, usually not exhibiting squamous cell metaplasia
- Carcinoma, adenosquamous, lung: Neoplasm composed of malignant squamous as well as nonsquamous, glandular elements

Metastases:

- Metastases from squamous cell carcinoma with its primary at another organ site are mostly multifocal and perivascular.

Comment: Primary squamous cell carcinoma at the tracheal bifurcation or in mainstem bronchi is one of the most lethal effects of cigarette smoking in humans and a major cause of human mortality (Greaves 2007). Primary carcinomas of the upper respiratory tract have been induced in rats by exposure to aldehydes and other irritant chemicals. There are few reports of experimentally induced squamous cell carcinomas in the airways of mice, but spontaneous development has not been reported.

(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; 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; St. Clair and Morgan 1992; Takahashi, Iwasaki, and Ide 1985; Yamamoto et al. 1989)

Adenocarcinoma (Figure 68): Larynx, Trachea, Bronchi, Bronchioles

Pathogenesis/cell of origin: Malignant transformation of respiratory epithelium.

Diagnostic features:

- Definitive evidence of origin from a conducting airway is required.
- Shows evidence of invasion of basement membrane or adjacent pulmonary structures
- Foci of mucinous cell differentiation may be present
- Papillary growth in early stages with central connective tissue stalk lined by cuboidal to columnar or pleomorphic epithelium
- Irregular tubular/glandular structures may be present
- Cytologic features of malignancy and possible evidence of stromal invasion and/or destruction of the airway wall

Differential diagnoses:

- Papilloma: Characterized by fairly uniform cuboidal cells without tissue invasion
- Squamous cell carcinoma: Growth pattern usually comprises cell clusters with central keratinization; in the absence of overt keratinization, there are distinct intercellular bridges.

Comment: Adenocarcinomas of the larynx, trachea, and bronchi are uncommon, with few reports of chemically induced tumors and in transgenic mice.

(Dixon and Maronpot 1991; Dungworth, Ernst, et al. 1992; Dungworth, Hahn, et al. 1992; Dungworth et al. 2001; Maronpot et al. 1991; Rehm, Ward, and Sass 1994)

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

Synonym(s): Carcinoma, clear cell; carcinoma, neuroendocrine.

Pathogenesis/cell of origin: Malignant transformation of the dispersed neuroendocrine cells in the tracheal epithelium.

Diagnostic features:

- Nodular thickening of tracheal wall with narrowing of lumen (present in the one case described thus far)
- Tumor cells separated into lobules and cords by delicate fibrovascular stroma
- Polygonal cells with distinct cell borders and abundant pale, finely granular cytoplasm
- Mitotic figures rare
- Invasion of all layers of tracheal wall
- Argyrophilic neurosecretory granules in cytoplasm by Grimelius silver stain

Differential diagnoses:

- Undifferentiated tumors: Histochemical, immunohistochemical, and ultrastructural methods necessary to make definitive diagnosis and to differentiate this tumor from a variety of undifferentiated tumors

Comment: Only one case of this tumor has been reported, which formed the basis for this description. Neuroendocrine proliferation is apparently extremely rare (Chandra, Riley, and Johnson 1991; Dungworth, Ernst, et al. 1992; Dungworth, Hahn, et al. 1992).

III. Terminal Bronchioles, Alveoli, and Pleura

Rats and mice lack well-developed respiratory bronchioles; thus, the transition from conducting to respiratory airways is abrupt (Tyler and Julian 1992). Each terminal bronchiole, alveolar duct, and associated alveoli makes up a pulmonary acinus (Mercer and Crapo 1992). Terminal bronchioles are lined by a mixture of columnar to cuboidal ciliated epithelium and noncilated (Clara) cells (Plopper and Hyde 1992). Type I pneumocytes cover the majority (93%–97%) of the alveolar surface and alveolar ducts; Type II cells, progenitors of Type I cells, occupy 3% to 5% of the alveolar epithelium and produce pulmonary surfactant. Detailed descriptions of the cells making up the bronchioles and the pulmonary acinus, their ability to differentiate, and their metabolic capabilities and distribution are described in detail elsewhere (Parent 1992; Plopper and Hyde 1992; Plopper and Dungworth 1987; Boorman and Eustis 1990; St. George et al. 1993). The visceral pleura and interstitial connective tissue of rodent lungs is relatively thin compared to domestic species and primates (Tyler and Julian 1992).

Alveolar Type I cells are more susceptible to injury than alveolar Type II cells, with the exception of situations in which the role of Type II cells in phospholipid metabolism makes them more vulnerable, for example, in phospholipidosis induced by amphiphilic cationic drugs (Philpot et al. 1977; Plopper and Dungworth 1987; Hook 1991). Ciliated bronchiolar epithelial cells are highly sensitive to direct-acting inhaled toxicants. High concentrations of metabolizing enzymes in Clara cells make them the most sensitive cells to inhaled or ingested chemicals requiring metabolic activation to be cytotoxic (Smith and Brian 1991). However, Clara cell enzymes may also decrease toxicity by deactivation of cytotoxic chemicals.

The location and microscopic appearance of induced lesions in lung parenchyma depends on a number of factors, including the route and duration of exposure, and the physical and chemical properties and concentration of the inhaled toxicant. Water solubility and reactivity are the most critical determinants of the depth of penetration and location of lesions from inhaled gases. The location of airway lesions induced by inhaled aerosols and particulates depends on particle size, shape, biopersistence, and chemical reactivity. The primary effects of particles less than 3 μm in aerodynamic diameter are usually seen in the alveolar ducts and alveoli. Lesions induced in lung parenchyma by inhalation of toxicants are most often distributed in a pattern consistent within pulmonary acinar units, consisting of terminal bronchioles, alveolar ducts, and alveoli. Many environmental toxicants induce lesions at the junction of terminal bronchioles and alveolar ducts (centriacinar region).

Spontaneously occurring pulmonary neoplasms in rodents are extensively described and illustrated in an ILSI Monograph (Rittinghausen et al. 1996a; Rittinghausen et al. 1996b), and in the goRENI database (http://www.goreni.org/). It can be difficult to clearly distinguish bronchiolo-alveolar hyperplasia from bronchiolo-alveolar adenoma or bronchiolo-alveolar adenoma from bronchiolo-alveolar carcinoma, and therefore, diagnosis may at times be arbitrary. Bronchiolo-alveolar adenoma can develop from bronchiolo-alveolar hyperplasia. With regard to histogenesis, the majority of papillary bronchiolo-alveolar tumors are considered by most investigators to represent less well–differentiated Type II cell tumors processing toward a malignant phenotype (Ohshima et al. 1985; Ward et al. 1985), whereas other investigators have demonstrated that in hamsters (Rehm and Lijinsky 1994) and

mice (Hicks et al. 2003), some papillary tumors may arise from Clara cells. Depending on the experimental design and/or carcinogen, specific histologic type of primary lung tumors can be induced selectively, or different types may be observed in a single animal.

A classification system for primary lung tumors in mice, which correlates closely with primary human lung tumor classification, was published recently by an international panel of lung cancer researchers (Nikitin et al. 2004). This panel recommended that when classifying experimentally induced primary lung tumors in rodents, the terms bronchiolo-alveolar and alveolar/bronchiolar be abandoned in favor of descriptive terms, namely, solid, papillary, mixed, and so on, to more closely correlate the morphological classification of experimental lung tumors in rodents with the morphologic classification of human lung tumors.

Description and classification of primary pleural neoplasms (mesothelioma) will be covered in the INHAND document on connective tissues.

A. Congenital Lesions: Terminal Bronchioles, Alveoli, Pleura

Congenital Cysts: Terminal Bronchioles, Alveoli, Pleura
Pathogenesis/cell of origin: Bronchioles, alveolar ducts, alveoli.

Diagnostic features:

- Severe dilatation of affected area.
- Little or no accompanying inflammation, fibrotic response, or proliferation in adjacent tissue.

Differential diagnoses:

- Emphysema: Inflammation present, multifocal, usually centriacinar distribution, loss of individual septa, but acinar structure is intact
- Dilatation secondary to blocked airway: Inflammation and evidence of blockage present
- Pulmonary squamous cysts: Large cysts filled with keratinized epithelium (see below)
- Epithelioma, cystic, keratinizing: Large cysts filled with keratinized epithelium (see below)
- Epithelioma, nonkeratinizing: Small, nodular lesion caused by filling of alveoli by squamous cells that have little or no evidence of keratinization

Comment: Isolated cystic spaces in lung parenchyma are rarely seen in rats and are of uncertain origin. These cysts are lined only by fibrous tissue, with no evidence of inflammation. Squamous cysts and cystic epitheliomas are described below under Proliferative Lesions.

Pulmonary Hypoplasia: Terminal Bronchioles, Alveoli
Pathogenesis/cell of origin: Bronchioles, alveolar ducts, alveoli, associated tissue.

Diagnostic features:

- Decreased development of alveoli
- Arrested differentiation of alveolar Type II pneumocytes into Type I cells (Brandsma et al. 1994)

Differential diagnoses:

- Type II pneumocyte hyperplasia: Normal architecture but alveoli are lined predominantly by Type II cells

Comment: Hypoplasia of lung parenchyma in neonatal rats induced by oral gavage exposure of dams to the herbicide Nitrofen has been used as an animal model for human pulmonary hypoplasia (Kimbrough, Gaines, and Linder 1974).

B. Epithelial Changes: Terminal Bronchioles, Alveoli, Pleura

Degeneration (Figure 69): Terminal Bronchioles, Alveoli
Pathogenesis/cell of origin: Epithelium lining bronchioles, alveolar ducts, alveoli.

Diagnostic features:

- Loss of cilia
- Epithelial blebbing or cytoplasmic vacuolation
- Rounding of normally cuboidal/columnar ciliated epithelium
- Pyknosis of nuclei

Differential diagnoses:

- Postmortem autolysis: Uniform dissolution of entire tissue section, with no change in organization or depth of cell layers
- Atrophy: Thinning of mucosal epithelium, but no inflammation or cell debris
- Necrosis: Pyknosis or karyorrhexis of nuclei, cytoplasmic eosinophilia, cellular swelling or shrinkage, exfoliation of cells, associated inflammation

Comment: Subtle degenerative changes in bronchiolar and alveolar epithelium are similar to those in the upper respiratory tract. Loss of cilia, rounding of normally cuboidal or columnar epithelial cells, and loss of normal apical blebs from Clara cells are typical early degenerative lesions seen in response to common urban pollutants such as ozone or nitrogen dioxide (Plopper and Dungworth 1987; Boorman and Eustis 1990; Haschek-Hock and Witchi 1991). Compounds requiring metabolism to produce their toxic effect, such as bromobenzene, carbon tetrachloride, or acetaminophen, induce degeneration and necrosis in Clara cells (Plopper and Dungworth 1987; Haschek-Hock and Witchi 1991).

Necrosis (Figure 70): Terminal Bronchioles, Alveoli
Pathogenesis/cell of origin: Epithelium lining bronchioles, alveolar ducts, and alveoli, and associated tissues.

Diagnostic features:

- Karyorrhexis or pyknosis of nuclei
- Sloughing of affected cells into the lumen

Differential diagnoses:

- Postmortem autolysis: Uniform dissolution of entire tissue section, with no change in organization or architecture
- Degeneration: Loss of cilia, epithelial vacuolation and bleb formation, increased intracellular spaces, loss of organization of cell layers

Comment: Necrosis and sloughing of bronchiolar epithelium and/or alveolar Type I cells release inflammatory mediators (Driscoll 1995) which, stimulate an acute inflammatory response, influx of macrophages, and proliferation of Type II cells and Clara cells to replace lost alveolar and bronchiolar epithelium.

Regeneration (Figure 71 and 72): Terminal Bronchioles, Alveoli

Pathogenesis/cell of origin: Epithelium lining the bronchioles, alveolar ducts, and alveoli.

Diagnostic features:

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

Differential diagnoses:

- Hyperplasia: Bronchiolar or alveolar epithelium is thickened because of increased numbers of cells, resulting in undulating, rugose epithelial surface of bronchioles and increased cellularity of alveoli, but alveolar architecture is still present (see Proliferative Lesions section of this document).
- Neoplasia: Expansile nodule usually protruding into bronchiolar lumen or compressing adjacent alveoli, with cellular atypia (see Proliferative Lesions section of this document).

Comment: Sequelae to loss of the epithelium lining the bronchioles are similar to those described above for upper airway epithelium, ranging from regeneration of the original epithelial cell types following a single insult, to luminal or peribronchiolar fibrosis (see below), or to squamous and/or goblet cell metaplasia, hyperplasia, and neoplasia following repeated injury. Loss of the epithelium lining terminal bronchioles, alveolar ducts, and alveoli stimulates replacement by proliferation of alveolar Type II cells and bronchiolar epithelium.

Specific types of alveolar epithelial regeneration in rodents are designated in the current literature as bronchiolization, bronchioloalveolar hyperplasia, or Type II cell hyperplasia (Schwartz et al. 1994; Dungworth, Hahn, and Nikula 1995). Lost alveolar epithelium is replaced by peripheral extension of bronchiolar epithelial cells into alveolar ducts and alveoli as well as metaplasia of Type II alveolar epithelium (Dungworth, Hahn, and Nikula 1995). Metaplasia and hyperplasia of bronchiolar and alveolar epithelia are described in detail and illustrated in the Proliferative Lesions section of this guide.

C. Intra-Alveolar Accumulations: Terminal Bronchioles, Alveoli, Pleura

Alveolar Macrophage Aggregation (Figures 73 and 74): Terminal Bronchioles, Alveoli

Synonyms: alveolar histiocytosis; alveolar phospholipidosis.

Pathogenesis/cell of origin: Alveolar ducts and alveoli.

Diagnostic features:

- Variable degrees of intra-alveolar aggregation of macrophages containing foamy cytoplasm
- Frequently observed spontaneously in the subpleural areas of aged animals (alveolar histiocytosis)
- Some macrophages in the aging lesions may contain hemosiderin
- Intra-alveolar accumulation of large numbers of lipid-containing macrophages (alveolar phospholipidosis) induced by alteration of endogenous lipid metabolism by certain cationic amphiphilic drugs

Differential diagnoses:

- Alveolar lipoproteinosis (see below): Alveoli contain particulate material and are filled with acellular, pale, PAS-positive lipoprotein and varying numbers of macrophages.
- Inflammatory response to inhaled material: Inhaled material is visible in macrophages.

Comment: The term "alveolar histiocytosis" is used to describe a continuum of lesions ranging from small foci of alveolar macrophages to mixtures of inflammatory cells in which macrophages predominate, to lesions in which fibrosis is a major feature (Dungworth, Ernst, et al. 1992). Foci of alveolar histiocytosis are commonly observed in untreated aged rats (Boorman and Eustis 1990; Brix et al. 2005). Intra-alveolar accumulation of large numbers of lipid-containing macrophages (alveolar phospholipidosis) is induced in rats by alteration of endogenous lipid metabolism by certain cationic amphiphilic drugs (Halliwell 1997; Hook 1991) or by hypophysectomy (Konishi and Higashiguchi 1996). Accumulation of macrophages in alveoli is one primary response to inhaled toxicants and is a prominent feature of the response to necrosis

of pulmonary parenchyma. Aggregates of macrophages may be present without accompanying increases in extracellular surfactant or progression to a prominent inflammatory lesion. Large numbers of macrophages migrate to the alveoli in response to chemotactic factors released through complement fixation and other processes stimulated by inhalation of cytotoxic materials (Warheit 1998). Phagocytosis of cytotoxic particles and subsequent death of macrophages releases other chemotactic factors for granulocytes, fibroblasts, and macrophages (Haschek-Hock and Witchi 1991).

Alveolar Lipoproteinosis (Figure 75): Terminal Bronchioles, Alveoli

Pathogenesis/cell of origin: Alveolar ducts, alveoli.

Diagnostic features:

- Pulmonary response to inhaled or instilled silica, characterized by filling of alveoli with acellular, PAS-positive pale eosinophilic material (lipoprotein) and varying numbers of macrophages

Differential diagnoses:

- Alveolar histiocytosis/phospholipidosis: Increased macrophages persist; drug-induced phospholipidosis will also affect other organs.
- Granulomatous inflammation: More cellular infiltrate than lipoproteinosis, with prominent macrophage component; acellular component not present
- Pulmonary edema: Solid pink, PAS-negative exudate with no cellular component
- Intra-alveolar fibrin or mucus: Laminar (fibrin) or pale bluish (mucin) exudates

Comment: Alveolar lipoproteinosis (Dungworth, Ernst, et al. 1992; Heppleston and Young 1972; Hook 1991) is readily induced in rats by repeated exposure to cytotoxic materials such as crystalline silica (quartz). This material has been identified by electron microscopy as the phospholipid surfactant material secreted by alveolar Type II cells. Macrophages filled with surfactant are relatively scarce in fully developed alveolar lipoproteinosis induced by inhalation of cytotoxic particles, whereas in drug-induced phospholipidosis, foamy macrophages persist (Dungworth, Ernst, et al. 1992).

Pigments, Dusts, Inert Materials (Figures 76–78): Terminal Bronchioles, Alveoli, Pleura

Pathogenesis/cell of origin: Bronchioles, alveolar ducts, alveoli.

Diagnostic features:

- Variably sized granular material visible in lumen and interstitium
- Found within macrophages or free in alveoli
- Increased numbers of macrophages
- Inflammatory response highly variable

Differential diagnoses:

- Alveolar histiocytosis: No evidence of pigment or other material
- Lipoproteinosis: Alveoli filled with acellular, pale, PAS-positive eosinophilic material and varying numbers of macrophages
- Inflammatory response to inhaled material: Inhaled material is visible in macrophages

Comment: The most frequently observed lung pigment in untreated aged rats is hemosiderin, a brown, iron-positive pigment usually found within perivascular or peribronchiolar alveolar macrophages. Lipofuscin may also be found within alveolar macrophages. Large hemoglobin crystals are occasionally observed free in the alveoli. Various exogenous dusts may be found in the lung parenchyma, usually within macrophages. Carbonaceous materials in diesel exhaust or other hydrocarbons retain their dark color through tissue processing and are visible in the alveoli. Other dusts, such as silica or talc, may be located by their birefringence with polarized light. The presence of relatively inert materials, such as corn oil vehicle accidentally instilled into the lung during gavage procedures, may cause minimal response and may be difficult to detect (Boorman and Eustis 1990).

D. Inflammation: Terminal Bronchioles, Alveoli, Pleura

Synonyms: Bronchiolitis, pneumonitis, pneumonia, pleuritis.

Pulmonary inflammation can be divided into lesions oriented primarily around alveoli and interstitium (alveolar/interstitial pattern) and lesions centered around or originating in the terminal airways (bronchioloalveolar pattern). This division is useful in understanding the pathogenesis and root causes of lesions in the lung parenchyma (Dungworth, Ernst, et al. 1992).

Inflammation, Acute Alveolar/Interstitial (Figure 79): Terminal Bronchioles, Alveoli, Pleura

Pathogenesis/cell of origin: Alveolar ducts and alveoli.

Diagnostic features:

- High concentration or severe toxicant induces edema, hemorrhage, and serofibrinous exudate involving the bronchioles and alveoli
- Mild toxicants may induce only a transient serous, fibrinous, or suppurative exudates.
- Variable microscopic evidence of a dosimetric gradient
- May or may not involve adjacent terminal bronchioles

Differential diagnoses:

- Primary cardiovascular event with secondary congestion and edema: Lack of inflammatory cellular infiltrate

Comment: Alveolar/interstitial inflammation is usually related to either a hematogenous insult or inhalation of very high concentrations of toxicants (especially gases) that do not permit a dosimetric gradient to be detected between bronchioles and alveoli

in distal portions of the acini (Dungworth, Ernst, et al. 1992). Inhalation of mildly toxic particles or vapors may induce only a transient serous, fibrinous, or suppurative exudate and/or increased numbers of macrophages in the alveoli. Hemorrhage and serofibrinous exudate predominate in acute diffuse alveolar damage caused by shocklike states, chemicals, and some acute systemic viral infections. Systemic bacterial infections affecting lung parenchyma via the bloodstream stimulate a suppurative alveolitis and perivascular infiltrate; viral infections may induce suppurative or mononuclear perivascular and alveolar infiltrates.

Inflammation, Chronic Interstitial (Figures 80 and 81): Terminal Bronchioles, Alveoli, Pleura

Pathogenesis/cell of origin: Connective tissue surrounding bronchioles, alveolar ducts, alveoli, and pleura.

Diagnostic features:

- Septal and interstitial fibrosis (see below)
- Accumulations of perivascular and peribronchiolar mononuclear inflammatory cells
- Hyperplasia of BALT

Differential diagnoses:

- Early connective tissue neoplasia: Uniform cell population infiltrating entire section

Inflammation, Acute Bronchioloalveolar (Figure 82): Terminal Bronchioles, Alveoli, Pleura

Synonym: Bronchopneumonia

Pathogenesis/cell of origin: Bronchioles, alveolar ducts, alveoli, associated tissue.

Diagnostic features:

- Serous, fibrinous, or suppurative intraluminal exudate in bronchioles and alveolar ducts, with variable extension into the alveoli
- Perivascular and alveolar congestion and edema
- Initial and most severe early changes are often observed in terminal bronchioles, alveolar ducts, and adjacent alveoli (centriacinar).
- Macrophages are a prominent feature of inflammatory infiltrates in alveolar duct and alveolar in lesions induced by particles and in resolving lesions in which epithelial necrosis has occurred.

Differential diagnoses:

- Other types of inflammation related to inhaled toxic materials (see Alveolar/Interstitial Inflammation, above)

Comment: Toxic or infectious agents sufficiently potent to induce necrosis of parenchymal cells stimulate an acute inflammatory response at the site of cell damage. The bronchioloalveolar pattern of inflammation is related in part to the fact that the terminal bronchioles, alveolar ducts, and adjacent alveoli are the sites of maximum deposition of inhaled small particles (Plopper and Dungworth 1987). Another factor is the fragility of the epithelium covering the terminal bronchioles and the more susceptible alveolar Type I cells lining the alveolar ducts and alveoli. Necrosis and ulceration of bronchiolar, alveolar duct, and alveolar epithelium stimulate predominantly intraluminal exudates, which will vary from serous to fibrinous to mucopurulent, depending on severity and time frame. Varying numbers of acute inflammatory cells and macrophages surround affected airways and adjacent blood vessels. Introduction of highly irritant materials into the lung parenchyma may induce localized acute necrosis with a pronounced suppurative inflammatory response (abscess), or a more slowly evolving granulomatous inflammatory response (see below). The suppurative inflammatory response to high concentrations of inhaled particulates such as titanium dioxide is much greater in rats compared to mice or hamsters (Bermudez et al. 2002; Bermudez et al. 2004). Pulmonary abscesses in rats in response to highly virulent pyogenic bacteria are very rare because of modern husbandry practices. Acute inflammation in the lung parenchyma in response to infection with pathogenic murine respiratory viruses is characterized by a variable amount of epithelial necrosis and a suppurative inflammatory exudate in bronchioles and alveoli. This inflammation is often followed by a perivascular and peribronchiolar infiltrate of lymphocytes and plasma cells, mucous (goblet cell) metaplasia in the affected bronchiolar epithelium (Buchweitz, Harkema, and Kaminski et al. 2007), and hyperplasia of BALT. The presence of numerous eosinophils in exudates and infiltrating the submucosa of airways, accompanied by perivascular infiltrates of lymphocytes and plasma cells, is suggestive of but not diagnostic for an immunologic or allergic basis for inflammatory lesions in lung parenchyma.

Inflammation, Chronic Bronchioloalveolar (Figures 83–91): Terminal Bronchioles, Alveoli, Pleura

Pathogenesis/cell of origin: Bronchioles, alveolar ducts, alveoli, associated tissue.

Diagnostic features:

- Perivascular and peribronchiolar mononuclear inflammatory cells
- Septal, interstitial, and pleural fibrosis (see below), mineralization, or osseous metaplasia
- Replacement of alveolar duct and alveolar epithelium with bronchiolar epithelium (metaplasia/bronchiolization)
- Bronchiolar and alveolar Type II epithelial cell hyperplasia
- Squamous or mucous (goblet) cell metaplasia of bronchiolar or alveolar epithelium
- Hyperplasia of BALT

Differential diagnosis:

- Primary interstitial inflammation: Lack of evidence of primary epithelial component; diffuse (vs. centriacinar) distribution

- Early hematopoietic, connective tissue, or epithelial neoplasia: Uniform cell population infiltrating entire section

Comment: Continuous exposure or inability to clear toxic or infectious agents from lung parenchyma leads to more severe and widespread cellular and interstitial inflammation in an attempt to eliminate or sequester the etiologic agent. In rodent lungs repeatedly exposed to cytotoxic or irritant materials, degeneration and necrosis of epithelial surfaces, and inflammatory infiltration and exudation induced by the initial exposure persist but may decrease in severity and evolve into interstitial fibrosis, metaplasia, and hyperplasia of damaged bronchiolar and alveolar epithelium (Kittel 1996; Buchweitz, Harkema, and Kaminski 2007). Bronchiolization occurs rarely as a spontaneous lesion in aged rats (Nagai 1994) but is a frequent feature of chronic inflammation induced in the centriacinar areas of the lungs of rats by repeated inhalation of toxicants (Brix et al. 2004). Bronchiolization was not observed in mice or hamsters exposed to similar concentrations of titanium dioxide (Bermudez et al. 2002; Bermudez et al. 2004) or carbon black (Elder et al. 2005). Hyperplasia and neoplasia of respiratory tract tissues are illustrated in the Proliferative Lesions portion of this publication. Continued severe epithelial hyperplasia, remodeling, and metaplasia may progress to malignant epithelial neoplasia in rats repeatedly exposed to high concentrations of relatively inert particles, or to very cytotoxic particles such as beryllium or nickel subsulfide. The significance of this carcinogenic response in rat lungs with regard to prediction of toxicity of inhaled particles in humans is the subject of controversy (Mauderly and McCunney 1996).

Inflammation, Granulomatous (Figures 92 and 93): Terminal Bronchioles, Alveoli, Pleura

Pathogenesis/cell of origin: Bronchioles, alveolar ducts, alveoli, associated interstitium.

Diagnostic features:

- Inflammatory lesions dominated by large numbers of macrophages, lymphocytes, plasma cells, and fibrosis
- Interlacing palisades of macrophages with abundant, finely granular cytoplasm (epithelioid cells) are the defining factor for a diagnosis of granulomatous inflammation.
- Multinucleated giant cells, formed from amitotic nuclear division or fusion of macrophages (Haley, 1991), are another hallmark of this entity.

Differential diagnoses:

- Early connective tissue or hematopoietic neoplasia (histiocytic sarcoma): Uniform cell population infiltrating entire section.

Comment: Granulomatous inflammation in the lung parenchyma may be caused by infectious agents or by inhalation of foreign materials such as metals or dusts (Haley 1991; Jones, Hunt, and King 1997). The type and severity of inflammatory response to foreign materials in the lung depends upon the physical characteristics and the amount of the material present. Pneumoconiosis is a generic term for the complex nonneoplastic granulomatous response of the lung to inhaled particles (Jones, Hunt, and King 1997; Roggli and Shelburne 1994). Crystalline silica, a material frequently used to study the pathogenesis of granulomatous inflammation in the lungs, has a range of cytotoxicity, which varies widely with surface chemistry (Parkes 1982; Roggli and Shelburne 1994). A frequent cause of granulomatous inflammation in the lungs of laboratory rats is intentional exposure via inhalation or intratracheal instillation to insoluble or poorly soluble respirable particles in toxicity studies. Crystalline silica (quartz), titanium dioxide, diesel exhaust, and beryllium are examples of materials that have been studied extensively in rats and mice (Boros 1978; Haley 1991; Bermudez et al. 2002; Bermudez et al. 2004). In toxicology studies, accidental injection of foreign materials into the lung is also an important cause of pulmonary lesions in rats. This may occur through improper gavage technique (Boorman and Eustis 1990) or secondary to excessive salivation and laryngeal paralysis caused by anesthesia with barbiturates (Gopinath, Prentice, and Lewis 1987). Highly irritant foreign bodies may induce severe pulmonary edema, a fatal acute suppurative bronchopneumonia, or a variable granulomatous inflammatory response, in which foreign material may be visible within well-developed granulomas (Dungworth, Ernst, et al. 1992). Brown-Norway rats develop a high background incidence of spontaneous granulomatous pulmonary lesions and are often used for the study of allergic airway disease. These lesions are characterized by interstitial infiltrates and alveolar exudates of eosinophils, macrophages, lymphocytes, and/or multinucleated giant cells and are exacerbated by oral administration of hexachlorobenzene (Michielsen et al. 2001) and by inhalation of trimellitic anhydride (Zhang et al. 2006). Eosinophilic crystalline pneumonia is an idiopathic granulomatous pneumonia of mice associated with a crystalline protein (Ym1) expressed in neutrophils and macrophages (Hoenerhoff, Starost, and Ward 2006; Ward et al. 2001). Ym1 protein has been implicated in host immune defense, tissue repair, and hematopoiesis.

Pulmonary Fibrosis (Figures 94–96): Terminal Bronchioles, Alveoli, Pleura

Pathogenesis/cell of origin: Connective tissue surrounding bronchioles, alveolar ducts, alveoli, and pleura.

Diagnostic features:

- Increased amount, abnormal location, or abnormal nature of collagen in lung parenchyma
- Special stains are useful to confirm the presence of excess or abnormal collagen.

- The terms fibrogenesis or fibroblastic response may be used to distinguish potentially reversible fibroblast proliferation with minimal cross-linking from irreversible pulmonary fibrosis with extensive cross-linking.

Differential diagnoses:

- Early connective tissue neoplasia: Uniform cell population infiltrating entire section; numerous mitotic figures, cellular atypia

Comment: Pulmonary fibrosis may be defined morphologically as an observable increase in amount or abnormal location of collagen in lung parenchyma, resulting in disruption of the normal lung architecture (Richards, Masek, and Brown 1991), or an abnormality in the nature of collagen in the lung (Haschek-Hock and Witschi 1991). Marked intraluminal fibrosis has been described in bronchioles of rats inhaling methyl isocyanate, a strong irritant. Fibrosis occurring as a sequel to lung injury has been reported most frequently in alveolar septa, interstitium, and pleura. Since fibrosis is considered an irreversible change, it is important to distinguish increases in amount of mature, cross-linked collagen in these areas from increases in septal or interstitial thickness resulting from edema or inflammation without substantial protein cross-linking. The term fibrogenesis has been used to describe potentially reversible fibroblast proliferation and minimal cross-linking of protein, as compared to true pulmonary fibrosis, in which extensive cross-linking has occurred and lesions are not reversible (Richards, Masek, and Brown 1991).

The critical event in the pathogenesis of pulmonary fibrosis is generally considered to be the release of potentially fibrogenic cytokines and fibronectin from macrophages activated as part of the inflammatory response to injury of lung parenchyma. Pulmonary fibrosis in laboratory rats is most frequently observed as part of a chronic inflammatory response to repeated injury to the lung parenchyma. However, severe acute lung injury from a single exposure may induce a relatively rapid fibrogenic response that may or may not be reversible. Diffuse interstitial fibrogenesis appeared rapidly following single exposures to bleomycin or BCNU (1,3-bis[2-chloroethyl]-1-nitroso-urea), but resolved within ninety days (Richards, Masek, and Brown 1991). However, repeated exposure to these toxicants induced irreversible pulmonary fibrosis.

The fibrogenic potential of inhaled particles varies widely; crystalline silica (quartz) is the classic model for induced pulmonary fibrosis in rats. Septal fibrosis is readily demonstrated in the lungs of rats repeatedly exposed to cytotoxic quartz particles and held for a prolonged postexposure period. Slowly progressive increases in interstitial collagen have been demonstrated during repeated exposures to ozone. Interstitial, subpleural, and pleural fibrosis may be prominent in rats chronically exposed via inhalation to respirable mineral fibers. Alveolar septal fibrosis occurred in rats exposed to high concentrations of pigmentary or ultrafine titanium dioxide or carbon black, but a lack of fibrosis in mice or hamsters exposed to the same concentrations of these materials (Bermudez et al 2002; Bermudez et al. 2004; Elder et al 2005). The issue of reversibility of interstitial fibrosis in postexposure periods following repeated exposures to ozone is not clear (Dungworth, Hahn, and Nikula 1995). Trichrome, Van Gieson's collagen stain, or other special stains are used to identify and quantify collagen in lung parenchyma. Biochemical and morphometric techniques are available to quantitatively determine collagen content in lungs of experimental animals (Richards, Masek, and Brown 1991).

Pyothorax: Pleura

Synonym: Suppurative pleuritis, pleurisy.

Pathogenesis/cell of origin: Mesothelium and connective tissue (visceral and parietal pleura) lining the thoracic cavity.

Diagnostic features:

- Accumulated suppurative exudate in thoracic cavity and pleura
- Usually accompanies similar inflammation in lung parenchyma

Differential diagnoses:

- Hydrothorax, hemothorax, chylothorax: Lack of inflammatory cells in fluid; lack of other evidence of a neoplastic lesion or edema fluid

Comment: Suppurative inflammation involving lung parenchyma may extend to involve visceral pleura, particularly if the exudate has a fibrinous component (Lopez 2007).

E. Abnormal Dilatation/Destruction of Alveoli: Alveoli

Pulmonary Acinar Ectasia (Figure 97): Alveoli

Pathogenesis/cell of origin: Alveolar ducts and alveoli.

Diagnostic features:

- Mild dilatation of alveoli and alveolar ducts, most frequently subpleural
- Seen most frequently in aged rats
- No morphologic evidence of alveolar wall destruction

Differential diagnoses:

- Alveolar emphysema: Evidence of alveolar wall destruction, chronic inflammatory infiltrates, centriacinar distribution
- Excess pressure at inflation of fixative: Diffuse distribution

Comment: This lesion has been classified as emphysema but is not consistent with the definition of alveolar emphysema, as there is no destruction of alveolar walls (Dungworth et al. 1992). The lesion may result in an increase in internal surface area of the lung and reduced gas-exchange efficiency (Mauderly

and Gillett 1992). It has been suggested that this is a "remodeling" of the lung associated with aging.

Alveolar Emphysema (Figure 98): Alveoli

Pathogenesis/cell of origin: Alveolar ducts and alveoli.

Diagnostic features:

- Abnormal enlargement of the air space distal to the terminal bronchiole accompanied by destructive changes of the alveolar septa
- Morphometric demonstration of increased lung volume, increased alveolar size, and decreased alveolar surface area confirm the diagnosis of emphysema. Constant pressure fixation of lungs and comparison to age-matched control animals is important
- Apparently does not occur as a spontaneous lesion in rats

Differential diagnoses:

- Pulmonary acinar ectasia: No evidence of inflammation, septal destruction, or centriacinar distribution
- Hyperinflation of lungs: Diffuse distribution, lack of destruction of septa, or inflammation

Comment: Emphysema can result from various injuries to the lung (Snider, Lucey, and Stone 1986). Intratracheal instillation of pancreatic elastase or papain is used to induce emphysema in rats for experimental purposes (Busch et al. 1984; Johansen, Pierce, and Reynolds 1971). Inhalation of cytotoxic particles, such as various nickel compounds, will induce emphysema by causing intense focal inflammation. Chronic exposure to cigarette smoke induces a mild centriacinar emphysema in rats and mice (March et al. 1999, 2006). Morphometric demonstration of increased alveolar size confirms the diagnosis of emphysema. Prevailing theories of pathogenesis of alveolar emphysema induced in rodents or humans by chronic exposure to irritants such as cigarette smoke include imbalances in protease-antiprotease levels, persistent inflammation with immunologic changes, and apoptosis (March et al. 2006; Wright and Churg 2007). Sendai virus infection in neonatal rats has been reported to induce alveolar emphysema by four months of age (Castleman 1992). The lungs of rats continue to grow until about ten months of age (Mauderly and Gillett 1992). The inflammation induced by viral infection in a rapidly growing lung may represent another way for emphysema to develop in the rat.

Atelectasis: Terminal Bronchioles, Alveoli

Pathogenesis/cell of origin: Alveolar ducts and alveoli.

Diagnostic features:

- The term refers to collapse of all or a portion of pulmonary alveoli
- Most often observed as a focal collapse of alveoli distal to airways obstructed by inflammatory exudates or tumors
- May be observed in alveoli distal to severe bronchiectasis (see above)
- Has been reported to occur in rats with rat coronavirus infection (Parker, Cross, and Rowe 1970)

Differential diagnosis:

- Artifactual collapse resulting from mishandling of tissues or failure to adequately inflate the lungs at tissue fixation: Diffuse lack of inflation, no evidence of inflammatory process

F. Vascular Changes: Terminal Bronchioles, Alveoli, Pleura

Hemorrhage, Pulmonary (Figure 99): Terminal Bronchioles, Alveoli, Pleura

Pathogenesis/cell of origin: Bronchioles, alveolar ducts, alveoli, associated tissue.

Diagnostic features:

- Presence of free blood in airways, around vessels, or in alveolar lumens
- Most frequently observed subpleurally when related to euthanasia with carbon dioxide

Comment: The use of 70% carbon dioxide for anesthesia, although doing so occasionally induces small areas of hemorrhage in alveoli, does not interfere with routine evaluation of rat lung morphology by light microscopy. The severity of intra-alveolar hemorrhages induced by inhalation of carbon dioxide is roughly proportional to its chamber concentration; 100% carbon dioxide will induce subpleural hemorrhages in rats (Renne et al. 2003). Pulmonary hemorrhage resulting from primary damage to endothelium has been described in rats experimentally poisoned with paraquat (Jones Hunt, and King 1997).

Congestion, Pulmonary (Figure 100): Terminal Bronchioles, Alveoli

Pathogenesis/cell of origin: Bronchioles, alveolar ducts, alveoli, associated tissue.

Diagnostic features:

- Dilatation of alveolar capillaries with widening of the alveolar septa

Differential diagnosis:

- Agonal changes: No evidence of antemortem inflammation or other damage
- Postmortem autolysis: Uniform dissolution of entire tissue section with no change in organization of cellular architecture

Comment: Diffuse dilatation of pulmonary capillaries is difficult to distinguish from agonal changes in the lungs of rats that die spontaneously, that are necropsied following a delay of twenty minutes or longer, or that are euthanized with carbon dioxide (Seaman 1987). This change is of no significance in the absence of other evidence of an antemortem lesion in the lungs or vasculature.

Edema, Pulmonary (Figures 100–102): Terminal Bronchioles, Alveoli

Pathogenesis/cell of origin: Bronchioles, alveolar ducts, alveoli, associated tissue.

Diagnostic features:

- Widening of perivascular and interlobar spaces (interstitial edema)
- Present in alveoli as pink-staining homogenous material
- Distinguish from fibrin with phosphotungstic acid hematoxylin (PTAH) stain
- Distinguish from extracellular pulmonary surfactant (proteinosis) by presence of numerous macrophages phagocytizing surfactant and particulate material in proteinosis
- Pleural effusion frequently accompanies pulmonary edema in rats.

Differential diagnosis:

- Alveolar lipoproteinosis: Alveoli filled with acellular, PAS-positive pale eosinophilic material and varying numbers of macrophages
- Fibrinous exudate: Laminar appearance
- Lymphedema: Presence of lymphocytes in exudates

Comment: Pulmonary edema results from either alteration in pulmonary hemodynamics or damage to the air–blood barrier in alveolar walls. Mild or early interstitial pulmonary edema may be detected by light microscopy as widening of the perivascular and interlobar spaces. If the primary cause of edema is direct damage to alveolar walls, or if interstitial edema is severe and prolonged enough to overwhelm the capability of the lymphatics to remove fluid, alveolar edema will result. Edema fluid in alveoli is visible as a pink-staining homogenous material in H&E-stained slides; the depth of pink staining is proportional to the protein content of the fluid. Detection of fibrin in alveolar fluid (indicative of a more severe lesion than simple edema) is aided by staining with PTAH. Pleural effusion of edema fluid occurs relatively frequently in rodent lungs; fluid readily passes through stomata in the thin pleura (Haschek-Hock and Witchi 1991). Critical assessment of the location and severity of alveolar edema in rat lungs fixed via intratracheal instillation of fixative is complicated by the instilled fixative changing the distribution of the edema fluid (Dungworth et al. 1992). Severe pulmonary edema may be acutely lethal in an organ as vital and as richly vascular as the lung.

Emboli, Pulmonary (Figure 103): Terminal Bronchioles, Alveoli

Pathogenesis/cell of origin: Pulmonary and bronchial vasculature.

Diagnostic features:

- Presence of visible fibrin or fatty thrombus in pulmonary vessel lumen
- Foreign material (hair or skin) may be visible in thrombus using polarized light
- Dual circulation (bronchial and pulmonary) prevents infarction unless systemic circulation is compromised
- Occurs as a complication of severe pneumonia

Differential diagnosis:

- Postmortem clot: No or few leucocytes present; no lamination indicating fibrin layers

Comment: In toxicologic studies, emboli consisting of fragments of hair or skin have been reported in pulmonary arteries or capillaries over 20% of rats after repeated intravenous injection (Kast 1996). These foreign bodies induce a suppurative or granulomatous response of variable magnitude and that may include multinucleated giant cells. Keratinized tissue (hair) is readily identifiable by its birefringence in polarized light.

Medial Hypertrophy of Pulmonary Arteries (Figures 104 and 105): Terminal Bronchioles, Alveoli

Pathogenesis/cell of origin: Pulmonary arteries.

Diagnostic features:

- Hypertrophy of smooth muscle and increased connective tissue in tunica media of normally muscular intra-acinar arteries
- Increased connective tissue in adventitia of normally muscular arteries
- Increased ratio of thickness of media/total diameter of intra-acinar arteries
- Presence of muscle in wall of normally nonmuscular peripheral arteries resulting from hyperplasia and hypertrophy of pericytes and intermediate cells
- Presence of visible internal elastic lamina in affected peripheral arteries

Differential diagnosis:

- Tangential section through vessel: Only part of vessel affected; other structures in section are cut tangentially, missing, or otherwise abnormal in shape

Comment: Chronic pulmonary hypertension has been induced in rats by chronic hypoxia, hyperoxia, and monocrotaline (Meyrick 1991; Rabinovitch 1991). The microscopic characteristics of the pulmonary vascular lesions include hypertrophy of smooth muscle and increased connective tissue in tunica media and increased

connective tissue in adventitia of normally muscular intra-acinar arteries. The internal elastic lamina is visible in affected arteries. Smooth muscle is present in the walls of normally nonmuscular peripheral arteries as a result of hypertrophy and hyperplasia of pericytes and intermediate cells. In affected rats, it is possible to quantify the changes in arterial wall thickness using special fixation and preparation techniques (Meyrick 1991).

Mineralization (Figures 106 and 107): Terminal Bronchioles, Alveoli, Pleura

Pathogenesis/cell of origin: Pulmonary vasculature, alveolar septa, associated connective tissue.

Diagnostic features:

- Linear mineralization of alveolar septa and pulmonary vessels, visible on H&E sections, confirmed with stains for minerals
- Frequently accompanied by macrophages and inflammation

Differential diagnosis:

- Postmortem autolysis: Uniform dissolution of entire section with no change in organization or architecture
- Inhaled bone or mineral: Recognizable histologically as bone; mineral will be within or surrounded by macrophages inflammation
- Osseous metaplasia: Recognizable histologically as bone

Comment: Focal mineralization of the walls of pulmonary arteries is frequently observed in older rats, usually with no apparent effect on the lung parenchyma. This material is subintimal but may affect the media in more severe cases (Dungworth, Ernst, et al. 1992). Mineralization of the alveolar walls and pulmonary vessels is present in severe cases of chronic nephropathy of aged rats and may be accompanied by increased macrophage aggregates or acute serous inflammation (Boorman and Eustis 1990).

Noninflammatory Pleural Effusions: Pleura

Synonyms: Hydrothorax, hemothorax, chylothorax.

Pathogenesis/cell of origin: Mesothelium and underlying connective tissue lining the thoracic cavity.

Diagnostic features:

- Accumulation of clear, serous fluid (hydrothorax, transudate), blood (hemothorax), or lymph (chylothorax) in thoracic cavity

Differential diagnoses:

- Pyothorax: Accumulation of suppurative exudate in the thoracic cavity
- Mesothelial hyperplasia or mesothelioma: Microscopic evidence of proliferation of mesothelium and/or underlying connective tissue. Effusions and mesothelial proliferation very often occur together.

G. Nonneoplastic Proliferative Lesions: Terminal Bronchioles, Alveoli, Pleura

Metaplasia, Mucous Cell (Figures 89 and 90): Terminal Bronchioles, Alveoli

Synonyms: Metaplasia, Goblet Cell

Pathogenesis/cell of origin: Clara cells and/or alveolar Type II epithelial cells which undergo mucous cell metaplasia.

Diagnostic features:

- Mucus-containing spaces lined predominantly by a single layer of mature mucous cells lining terminal bronchioles or alveoli
- Very low mitotic activity
- PAS/Alcian blue-staining enhances identification of mucous cells and mucus
- Usually accompanied by chronic inflammation and fibrosis
- Proliferation of other epithelial elements as described for bronchiolo-alveolar hyperplasia may be present
- Background of normal bronchiolo-alveolar architecture retained to various degrees, depending on the degree of associated inflammation and fibrosis

Differential diagnoses:

- Hyperplasia, bronchiolo-alveolar: Absence or insignificant amounts of mucous cells and intraluminal mucus (see Comment)
- Adenoma, bronchiolo-alveolar: More densely cellular nodular mass. Papillary to solid tumor formations within alveoli and alveolar ducts obscure the normal alveolar architecture. Inconspicuous or absent inflammatory components within the tumor
- Carcinoma, acinar: Well-developed glandular structures with invasion of basement membranes

Comment: Mucous cell metaplasia usually occurs in lungs of animals chronically exposed to airborne irritants and is frequently associated with foci of bronchiolo-alveolar hyperplasia. Small foci of mucous cell metaplasia may occur incidentally in untreated mice. Exaggerated mucous cell metaplasia has been observed as a rare lesion at the bifurcations of medium-sized airways in mice exposed to cobalt sulfate aerosols. Mucous cell metaplasia may be associated with squamous cell metaplasia.

(Boorman and Eustis 1990; Dungworth, Ernst, et al. 1992; Dungworth et al. 2001; Faccini, Abbott, and Paulus 1990; Frith and Ward 1988; Kittel 1996; Mohr et al. 1990; Nagai 1994; Pour et al. 1976; Rehm et al. 1991; Rehm, Ward, and Sass 1994)

Metaplasia, Squamous Cell (Figure 87): Terminal Bronchioles, Alveoli

Pathogenesis/cell of origin: Clara cells and/or alveolar Type II cells that undergo squamous cell metaplasia.

Diagnostic features:

- Replacement of alveolar epithelium by squamous cells, which may contain only keratohyaline granules, or be highly keratinized
- More commonly multifocal
- Orderly progression from basal cells to keratinizing surface epithelium in areas of keratinization
- Rarely slight nuclear polymorphism and cellular atypia; occasional mitotic figures may be present.
- Usually associated with Clara, mucous, and/or ciliated cells

Differential diagnoses:

- Carcinoma, squamous cell: Normal pulmonary architecture disturbed. Mostly singular nodule. Presence of features of malignancy such as cellular or nuclear atypia, or frequent mitoses, or invasive growth, or metastases
- Hyperplasia, bronchiolo-alveolar: Little or no squamous cell component
- Cyst, keratinizing (rats only): May be several centimeters in diameter and filled with large amounts of keratin. Has thin, uniform cyst wall composed of well-differentiated, flattened squamous epithelium undergoing orderly maturation
- Epithelioma, cystic, keratinizing (rats only): Expansile nodule with central keratinization and necrosis, which effaces pulmonary parenchyma. Thick, irregular, complex cyst wall that lacks of orderly maturation and increased numbers of mitotic figures
- Epithelioma, nonkeratinizing (rats only): Expansile cellular nodule lesion composed of squamous epithelial cells that obscures and distorts alveolar architecture. Cells near the periphery of the mass have small, round to oval nuclei and little cytoplasm (basaloid appearance); centrally located cells have more abundant, finely granular eosinophilic cytoplasm and inconspicuous intercellular bridges. Little or no evidence of keratin

Comment: Different usages of hyperplasia, metaplasia and transdifferentiation have been applied for changes in cell populations lining damaged bronchiolo-alveolar regions. Metaplasia is used to denote a mature cell type not normally resident at the junction of terminal bronchioles and alveolar ducts, namely, squamous or mucous cells. Squamous metaplasia may mimic squamous cell tumors in rats and may occur in rats fed vitamin A–deficient diets, in animals with subacute to chronic infectious pneumonias, and in lungs of rats chronically exposed to irritants in inhalation studies.

(Boorman and Eustis 1990; Dungworth, Ernst, et al. 1992; Dungworth et al. 2001; Faccini, Abbott, and Paulus 1990; Frith and Ward 1988; Mohr et al. 1990; Pour et al. 1976; Rehm et al. 1991; Rehm, Ward, and Sass 1994)

Hyperplasia, Bronchiolo-Alveolar (Figures 108 and 109): Terminal Bronchioles, Alveoli

Synonym(s): Hyperplasia, bronchiolar/alveolar; hyperplasia, Type II cell; bronchiolization.

Modifier: Alveolar; bronchiolar (bronchiolization); mixed.

Pathogenesis/cell of origin: Proliferation of alveolar Type II cells and/or bronchiolar ciliated respiratory or secretory cells.

Diagnostic features:

- Solitary or multiple, segmental (cone-shaped) foci of increased cellularity
- Lack of strongly convex or spherical border
- Bronchiolo-alveolar architecture still detectable
- Epithelial cells dominant and are the cause of hypercellularity
- Epithelial cells usually single layered

Depending on the situation in which this change arises (see comment), classification into one of the following histologic types is often possible.

Alveolar:

- Round to oval or cuboidal often/mostly hypertrophic alveolar Type II cells with abundant eosinophilic cytoplasm prominently outlining alveolar walls. Increased basophilia may be associated with cuboidal cell shape.
- Cytoplasm may be vacuolated.
- Cells form a single layer that is contiguous throughout the area of hyperplasia.
- Areas of alveolar hypercellularity may be single or multiple, round, or cone-shaped if localized subpleurally. Peripheral margins are indistinct.
- Associated with terminal airways by extension of peripheral growth
- Formation of solid cell clusters and papillary projections marks transition toward neoplasia
- May be associated with influx of alveolar macrophages

Bronchiolar (bronchiolization):

- Alveolar walls are lined by cuboidal to tall columnar or possibly pleomorphic cells that may have bronchiolar epithelial cell differentiation, for example, cilia, resemble Clara cells (apical protrusions), or presence of mucous granules or eosinophilic globules.
- May be associated with squamous cell metaplasia
- Normal resident alveolar Type II and Type I cells may be interspersed.
- Cells form a single layer or focal tufts of cells that may appear pseudostratified.
- Areas of hyperplasia centered on terminal bronchioles, but in some planes of section the connection between the foci of bronchiolization and the terminal bronchiole may not be observed.
- Peripheral borders indistinct, as with alveolar cell hyperplasia

- Formation of increased cellular atypia, acini contiguously lined by single or stratified cells, and basement membrane invasion mark neoplasia.

Mixed type:

- Various proportions of bronchiolar and alveolar types of hyperplasia

Special techniques for diagnosis: Routine light microscopy may not permit definitive identification of the cells composing these lesions as bronchiolar (especially Clara cells) or alveolar Type II cells, and other techniques such as immunohistochemistry may have to be used. The most reliable immunohistochemical markers are the 16 kDa protein (CC16) for Clara cells and surfactant apoprotein A or C for Type II cells. Positive immunoreactivity for surfactant apoprotein A and negative immunoreactivity for CC16 provide good presumptive evidence for lesions of Type II cell origin.

Differential diagnoses:

- Adenoma, Bronchiolo-Alveolar: Densely cellular rounded nodule. Obscuring of the normal alveolar architecture by solid to papillary tumor formations within alveoli and alveolar ducts
- Carcinoma, Bronchiolo-Alveolar: Increased cellular atypia or invasion or destruction of adjacent pulmonary structures
- Metaplasia, Mucous Cell: Consists predominantly of mucous cells
- Metaplasia, Squamous Cell: Consists predominantly of squamous cells
- Carcinoma, Acinar: Well-developed glandular structures with invasion of basement membranes

Comment: Bronchiolo-alveolar hyperplasia, by convention, refers to epithelial hyperplasia distal to terminal bronchioles affecting alveoli immediately adjacent to the alveolar ducts. Bronchiolar-alveolar hyperplasia in the lungs of SPF control rats or those fed nonpneumotoxic agents is often unaccompanied by a significant inflammatory cell components. In rats exposed to airborne or intratracheally instilled irritants, however, bronchiolo-alveolar hyperplasia and inflammation are often inseparable.

Lining of alveolar ducts and alveoli by bronchiolar epithelial cell types is commonly called bronchiolization. This condition most often occurs in mice exposed to airborne irritants or by intratracheal instillation and is a late sequela to Sendai virus infection. The relative contribution of distal growth of preexisting bronchiolar epithelium and metaplasia (transdifferentiation) of alveolar Type II cells is not clear and probably varies according to causal agent. In the lungs of control mice, the separation of bronchiolization and alveolar Type II cell hyperplasia is often fairly clear, whereas in chronically inflamed lungs of mice, there is much more likely to be mixed bronchiolo-alveolar hyperplasia.

The distinction between bronchiolo-alveolar hyperplasia and bronchiolo-alveolar adenoma is often difficult. Arbitrary guidelines have been suggested, such as obliteration of three or more contiguous alveolar spaces as the criterion for an adenoma. This criterion is not as easy to apply in practice with the lung as with solid organs.

Eosinophilic globules (eosinophilic intracytoplasmic proteinaceous accumulations) may be a prominent feature of all types of hyperplasia and may also be found in nonhyperplastic cell populations. They are generally believed to represent dilated endoplasmic reticulum containing proteinaceous material.

(Belinsky et al. 1992; Boorman and Eustis 1990; Dungworth, Ernst, et al. 1992; Dungworth et al. 2001; Ernst, et al. 1996; Foley et al. 1991; R. A. Miller and Boorman 1990; Mohr et al. 1990; Nettesheim and Szakal 1972; Ohshima et al. 1985; Pack, Al-Ugaily, and Morris 1981; Rehm and Kelloff 1991; Rehm et al. 1991; Rehm, Ward, and Sass 1994; Rehm et al. 1988; Singh et al. 1985; ten Have-Opbroek 1986; Ward et al. 1985; Witschi 1986)

Hyperplasia, Mesothelium (Figure 110): Pleura

Pathogenesis/cell of origin: Mesothelium lining thoracic cavity.

Diagnostic features:

- Focal or diffuse increase in layers of pleural mesothelium from normal one- to two-cell thickness, accompanied by inflammatory cells and an increase in underlying connective tissue
- Mesothelial cells are cuboidal with prominent nucleoli, abundant cytoplasm compared to flattened normal mesothelial lining cells (Everitt et al. 1994).
- Hyperplastic mesothelial response to instilled particulates is reported to be most severe in parietal pleura lining diaphragmatic surface (Everitt et al. 1997).

Differential diagnoses:

- Mesothelioma: Evidence of cellular atypia and/or invasion of adjacent lung parenchyma or thoracic wall
- Pleural fibrosis and associated inflammation: Presence of interlacing bundles of spindle cells with some evidence of collagen formation and associated inflammatory cells; no evidence of proliferating mesothelium

Comment: Considerable information is available on the morphology and biologic potential of hyperplastic and inflammatory pleural responses to instilled particulates in rodents (Everitt et al. 1994, 1997).

Pulmonary Keratinizing Cyst (Rats only) (Figure 111): Alveoli

Pathogenesis/cell of origin: Alveolar or bronchiolar epithelial cells which have undergone squamous metaplasia.

Diagnostic features:

- May be several centimeters in diameter and filled with large amounts of keratin
- Thin, uniform cyst wall composed of well-differentiated, flattened squamous epithelium undergoing orderly maturation is a key distinguishing feature
- Compression of adjacent lung is usually inconspicuous
- Mitoses rarely seen or absent

Differential diagnosis:

- Metaplasia, squamous cell: Tends to be multifocal. Small size. Preserves the normal pulmonary architecture
- Epithelioma, nonkeratinizing: Expansile cellular nodule lesion composed of squamous epithelial cells that obscures and distorts alveolar architecture. Cells near the periphery of the mass have small, round to oval nuclei and little cytoplasm (basaloid appearance); centrally located cells have more abundant, finely granular eosinophilic cytoplasm and inconspicuous intercellular bridges; little or no evidence of keratinization
- Epithelioma, cystic, keratinizing: Expansile nodule with central keratinization and necrosis that effaces pulmonary parenchyma. Thicker, irregular, and more complex cyst wall that lacks of orderly maturation and increased numbers of mitotic figures
- Carcinoma, squamous cell: Destruction of the basement membrane. Cellular atypia and disorientation, frequent mitoses. Presence of other signs of malignancy, including invasion into the interstitium, lymphatics, blood vessels, or pleural surfaces, or distant metastasis

Comment: The literature contains a variety of names for a series of large squamous cystic lesions observed in the lungs of rats following chronic exposure to high concentrations of particulate material. These lesions were given morphologic classifications, including squamous metaplasia, squamous cysts, squamous epithelioma, and squamous cell carcinoma. The exact nature of the squamous cell proliferation in these lesions is uncertain. Because of the location and pattern of growth within the pulmonary parenchyma, it does not appear to be simple squamous hyperplasia. Across a variety of experimental studies, there is a continuum from a cystic squamous lesion to invasive squamous cell carcinomas. Boorman, Morgan, and Uraih (1996) presented the results and conclusions of an expert panel of toxicologic pathologists from Europe and North America convened to review and set diagnostic criteria for these pulmonary cystic squamous lesions in rats. The criteria for diagnosis of pulmonary keratinizing cyst and pulmonary cystic keratinizing epithelioma (see below) presented in this document are based on the published results of the workshop. Clear distinction between squamous cyst, benign squamous cell tumor, and well-differentiated squamous cell carcinoma is sometimes arbitrary, as is the distinction between exaggerated squamous metaplasia and benign keratinizing cystic tumor. These large cystic, squamous lesions have not been reported in mice.

H. Neoplastic Proliferative Lesions: Terminal Bronchioles, Alveoli, Pleura

Epithelioma, Cystic, Keratinizing (Rats only) (Figure 112): Alveoli

Synonym(s): Tumor, squamous cell, keratinizing, cystic, benign; epithelioma, squamous; cyst, squamous.

Pathogenesis/cell of origin: Neoplastic proliferation of squamous metaplasia (transdifferentiation) and neoplastic transformation of alveolar epithelium and/or Clara cells.

Diagnostic features:

- Cyst wall consists of a mixture of squamous epithelium with numerous mitoses, lacking orderly maturation in some areas, and a thin layer of flattened squamous epithelium adjacent to the pleura or interstitium surrounding major airways and vessels
- Growth occurs mostly by peripheral extension into the alveolar spaces, giving a rough or cobblestoned appearance at the periphery of the lesion
- Basal cells are disorganized in foci of active cellular proliferation and may have increased numbers of mitotic figures.
- Usually large amounts of keratin and necrotic tumor tissue in the center of the tumor mass

Differential diagnoses:

- Metaplasia, squamous cell: Tends to be multifocal; preserves the normal pulmonary architecture
- Pulmonary keratinizing cyst: May be several centimeters in diameter and filled with large amounts of keratin. Has thin, uniform cyst wall composed of well-differentiated flattened squamous epithelium undergoing orderly maturation; compression of adjacent lung parenchyma is usually inconspicuous; mitoses rare or absent.
- Epithelioma, nonkeratinizing (rats only): Expansile cellular nodule lesion composed of squamous epithelial cells that obscures and distorts alveolar architecture. Cells near the periphery of the mass have small, round to oval nuclei and little cytoplasm (basaloid appearance); centrally located cells have more abundant, finely granular eosinophilic cytoplasm and inconspicuous intercellular bridges. Little or no evidence of keratin.
- Carcinoma, squamous cell: Destruction of the basement membrane. Cellular atypia and disorientation, frequent mitoses. Additional signs of malignancy may be present, such as invasion into the interstitium,

or lymphatics, or blood vessels, or pleural surfaces, or distant metastasis.

Comment: See Pulmonary Keratinizing Cyst (above).

(Boorman 1985a; Boorman 1985b; Boorman 1985c; Boorman and Eustis 1990; Boorman, Morgan, and Uraih 1996; Dungworth, Ernst, et al. 1992; Dungworth, Hahn, et al. 1992; Kittel et al. 1993; Mohr et al. 2006; Mohr et al. 1990; Rittinghausen et al. 1992; Rittinghausen and Kaspareit 1998)

Epithelioma, Nonkeratinizing (Figure 113): Alveoli

Synonym(s): Tumor, squamous cell, nonkeratinizing, benign.

Pathogenesis/cell of origin: Neoplastic proliferation of squamous metaplasia (transdifferentiation) and neoplastic transformation of alveolar epithelium and/or Clara cells.

Diagnostic features:

- Small, nodular lesion caused by filling of alveoli by squamous cells
- Cells toward the periphery of the alveolar accumulations have small, round to oval nuclei and little cytoplasm (basaloid appearance)
- Centrally located cells tend to have more abundant, finely granular eosinophilic cytoplasm and inconspicuous intercellular bridges.
- Mitoses rarely seen
- Little or no evidence of keratinization

Differential diagnoses:

- Metaplasia, squamous cell: Multifocal, small size, preserves the normal pulmonary architecture
- Pulmonary keratinizing cyst: May be several centimeters in diameter and filled with large amounts of keratin. Has thin, uniform cyst wall composed of well-differentiated, flattened squamous epithelium undergoing orderly maturation; compression of the adjacent lung parenchyma is usually inconspicuous; mitoses rare or absent
- Epithelioma, cystic, keratinizing: Expansile nodule with central keratinization and necrosis, which effaces pulmonary parenchyma. Thicker, irregular and more complex cyst wall that lacks orderly maturation and increased numbers of mitotic figures
- Carcinoma, squamous cell: Destruction of the basement membrane, or cellular atypia or disorientation, or frequent mitoses. Or presence of other signs of malignancy, such as invasion into the interstitium, lymphatics, blood vessels, or pleural surfaces, or distant metastasis

Comment: The nonkeratinizing squamous cell tumor is distinguished from the keratinizing cystic epithelioma because, although rare, it appears as a distinct entity and has a malignant counterpart.

(Boorman, Morgan, and Uraih 1996; Dungworth et al. 1992; Mohr et al. 1990; Rittinghausen et al. 1992)

Adenoma, Bronchiolo-Alveolar (Figure 114): Terminal Bronchioles, Alveoli

Synonym(s): Adenoma, Type II cell; adenoma, pulmonary.

Modifiers: Solid; papillary; mixed.

Pathogenesis/cell of origin: It is generally believed that solid tumors are composed of cells expressing alveolar Type II cell features and are therefore considered benign alveolar Type II cell tumors. Papillary tumors, however, are considered either to be less well-differentiated Type II cell tumors progressing toward a malignant phenotype or to be of Clara cell origin. Clara cell tumors are rare in rats and mice. Clara cell tumors have been reported in transgenic mice made expressly to target the Clara cells. Most papillary tumors in mice arise from alveolar Type II cells.

Diagnostic features:

- Frequently located at the lung periphery and usually small in size (in mice less than 3–4 mm in diameter)
- Well-circumscribed areas of high epithelial cell density, usually with strongly convex border
- Underlying alveolar architecture obscured to various degrees
- Sharp demarcation from the surrounding tissue
- Neoplastic epithelial cells relatively uniform
- Mitotic figures are rare or absent
- Small foci of mild atypia may be present. In these foci, cells tend to have a higher degree of pleomorphism, and the number of mitoses is slightly increased
- Occasionally extend into adjacent bronchioles

Solid:

- Alveolar spaces obliterated by proliferating round to oval cells. Frequently these solid areas are surrounded by alveoli lined by hyperplastic alveolar Type II cells; namely, there might be no sharp demarcation between the tumor and normal parenchyma.
- Cells usually have abundant eosinophilic cytoplasm that may appear granular or vacuolated. Cell nuclei are usually round to oval.
- Mitotic figures are rare or absent.
- May extend into bronchiole through the alveolar duct.
- Compression is frequently observed.

Papillary:

- Composed predominantly of delicate papillary structures lined by cuboidal to columnar cells that may be deeply basophilic
- Regular pattern (lack of distortion and focal variation in cell appearance)

- Tumors may show prominent tubular profiles, namely, elongated lumina surrounded by cuboidal cells, depending on orientation of the histologic section.
- Papillary structures sharply demarcated from surrounding alveolar parenchyma
- May be associated with peripheral alveolar hyperplasia
- Large, sometimes foamy, macrophages may fill spaces between tumor cells.
- Mitotic rate and the degree of cellular pleomorphism are usually low. There is no invasion and destruction of adjacent tissue.

Alveolar (rats only):

- Alveolar (glandular) growth pattern: Cuboidal or columnar cells that enclose a central lumen. Only slight cellular atypia. Often ovoid nuclei

Tubular (rats only):

- Tubular growth pattern: Prominent tubular profiles, that is, elongated lumina

Mixed:

- In rats: Alveolar, tubulo-papillary, as well as solid areas in the same neoplasms
- In mice: Papillary and solid areas may exist in the same neoplasm.

Differential diagnoses:

- Hyperplasia, bronchiolo-alveolar (bronchiolar or mixed): Less well defined, segmental (cone-shaped) lesion with apex at terminal bronchiole. Lining of alveolar ducts and alveoli distal to bronchiole by a single layer of epithelial cells without significant distortion of pulmonary architecture unless accompanied by scarring and/or inflammation. There is an absence of prominent papillary proliferations or complete filling of alveoli by epithelial cells.
- Hyperplasia, bronchiolo-alveolar (alveolar): Alveolar walls prominently outlined by single, continuous layer of hyperplastic alveolar Type II cells. No significant obliteration of contiguous alveolar spaces by areas of solidly packed cells or papillary fronds. No compression of the adjacent lung parenchyma
- Papilloma of bronchiole: May be morphologically identical to papillary bronchiolo-alveolar adenoma of lung parenchyma. A diagnosis of bronchiolar papilloma should be made only if there is clear evidence that the tumor arose from bronchiolar epithelium.
- Carcinoma, bronchiolo-alveolar: Increased cellular pleomorphism or atypia or mitotic figures; effacement or distortion of pulmonary parenchyma, possibly with local invasion or metastasis.

Comment: It can be difficult to clearly distinguish bronchiolo-alveolar hyperplasia from bronchiolo-alveolar adenoma or bronchiolo-alveolar adenoma from bronchiolo-alveolar carcinoma, and therefore, diagnosis may at times be arbitrary. Bronchiolo-alveolar adenoma may develop from bronchiolo-alveolar hyperplasia. There are no reports on malignant solid carcinomas in mice. Compression is present as an artifact in immersion-fixed lung but is not a significant feature in lungs fixed at near normal volume by intratracheal infusion of fixative. Eosinophilic globules may be a prominent feature associated with bronchiolo-alveolar adenoma.

(Beer and Malkinson 1985; Belinsky et al. 1992, 1991; Boorman 1985a; Boorman 1985b; Boorman 1985c; Boorman, Morgan, and Uraih 1996; Boorman and Eustis 1990; Branstetter and Moseley 1991; Breeze and Wheeldon 1977; Dixon et al. 1991; Dixon and Maronpot 1991; Dungworth, Ernst, et al. 1992; Dungworth, Hahn, et al. 1992; Dungworth et al. 2001; Foley et al. 1991; Gunning, et al. 1991; Gunning, Stoner, Goldblatt 1991; Gunning, Goldblatt, and Stoner, 1992; Heath, Frith, and Wang 1982; Malkinson 1989; Maronpot et al. 1991; R. A. Miller and Boorman 1990; Mohr et al. 1990; Ohshima et al. 1985; Palmer 1985; Palmer and Grammas 1987; Plopper et al. 1983; Pour et al. 1976; Rehm et al. 1991; Rehm and Ward 1989; Rehm et al. 1991; Rehm, Ward, and Sass 1994; Rehm et al. 1988; Reznik-Schüller and Reznik 1979; Rittinghausen et al. 1992; Rittinghausen et al. 1996a; Rittinghausen et al. 1996b; Schüller 1987; Schüller 1990; Singh et al. 1985; Thaete et al. 1987; Thaete and Malkinson 1990, 1991; Thaete, Nesbitt, and Malkinson 1991; Ward et al. 1983; Ward and Rehm 1990; Ward et al. 1985; Witschi 1986; Yamamoto et al. 1989)

Carcinoma, Bronchiolo-Alveolar (Figure 115): Terminal Bronchioles, Alveoli

Synonym(s): Adenocarcinoma, pulmonary; adenocarcinoma, bronchiolo-alveolar (see comment below).

Pathogenesis/cell of origin: Bronchiolo-alveolar carcinomas may originate from either alveolar Type II or Clara cells but are generally considered to arise from alveolar Type II cells.

Diagnostic features:

- Irregular nodular growth, moderately well to poorly circumscribed neoplasms (in mice often greater than 3–4 mm in diameter). May occupy an entire lobe
- Architectural distortion and variation in appearance and organization of tumor cells from one region to another
- Increased mitotic activity

Rats:

- Alveolar (glandular) growth pattern: Cuboidal to columnar cells forming glandlike structures.

- Papillary growth pattern: Cuboidal to columnar or pleomorphic cells, arranged as papillary structures supported by a connective tissue core
- Tubular growth pattern: Prominent tubular (elongated) profiles in section
- Solid growth pattern: Closely apposed round cells without spaces between them
- Mixed growth pattern: Glandular, papillary, as well as solid areas occurring in the same neoplasms

Mice:

- Papillary growth pattern: Cuboidal to columnar or pleomorphic cells, arranged as papillary structures supported by a connective tissue core; cytoplasm may contain glycogen and/or neutral lipids.

Rats and Mice:

- Tumors may exhibit areas of increased cytoplasmic basophilia and atypia indicating local expansion of less differentiated tumor cells.
- Frequently associated with influx of macrophages into the tumor and in adjacent alveoli
- Large tumors may have areas of necrosis, hemorrhage, cholesterol clefts, fibrosis (especially at subpleural localization), and obliterating fibrosis of bronchioles.
- Indication of malignancy, such as destruction of parenchyma, invasion of bronchiolar walls, interstitial tissues and/or pleura, and/or dissemination through lymphatics, airspaces, and/or distant metastases
- Advanced stages of malignancy and invasion (e.g., to pleura) frequently associated with marked cellular pleomorphism (spindle-shaped to round atypical cells), desmoplasia, increased mitotic rate
- Areas of squamous cell metaplasia may be found.

Differential diagnoses:

- Adenoma, bronchiolo-alveolar: No invasive growth into pulmonary vessels or extrapulmonary parenchyma, no metastases. No or only slight cellular pleomorphism or atypia
- Carcinoma, acinar: Predominantly glandular/acinar pattern representing tumor cells that utilize preexisting alveolar walls. Composed of cuboidal to columnar or pleomorphic cells without distinguishing features, or, more commonly, mixed with ciliated cells, or mucous cells. Large portions or the entire tumor may show differentiation in favor of single cell type, for example, mucinous adenocarcinoma.
- Carcinoma, adenosquamous or carcinoma, squamous cell: Tumor shows areas of malignant squamous cells or is composed entirely of characteristically squamous cell carcinoma
- Mesothelioma, malignant: Differentiation of pleural spread of papillary bronchiolo-alveolar carcinoma from malignant mesothelioma of epithelioid type can be difficult. Metastases of bronchiolo-alveolar carcinomas (to pleura, etc.) frequently are very anaplastic and may be misdiagnosed as malignant mesotheliomas of mesenchymal type.
- Metastases: Metastases of primary adenocarcinoma from other organs to the lung are primarily perivascular and multifocal. Careful search may reveal tumor emboli in pulmonary vasculature.

Comment: Bronchiolo-alveolar adenomas and carcinomas are the most common spontaneous and chemically induced lung tumors in rats and mice. The histogenesis of these tumors is controversial. Tumors with histologic features of Type II alveolar cells, bronchiolar Clara cells, or both of these cell types have been described, but they are generally considered to arise from alveolar Type II cells. Because the histogenesis of these lung tumors is debatable, it has been the convention to classify lung tumors originating in the alveolar/bronchiolar region as alveolar/bronchiolar adenomas or carcinomas.

Bronchiolo-alveolar tumors are considered to represent a morphologic and biologic continuum, and as such, it can be sometimes difficult to distinguish benign and malignant tumors. As described above, several distinct histological types of bronchiolo-alveolar tumors are recognized. However, it may also be difficult to separate these histologic types. When evaluating toxicologic studies, the pathologist should consider when and if it is appropriate to make distinctions based on the perceived histologic type. As stated above, because of the uncertain histogenesis, it may be more appropriate to classify lung tumors originating in the alveolar/bronchiolar region of the lung in rats and mice as bronchiolo-alveolar tumors.

Although widely in use as described above in the veterinary literature, the terms bronchioloalveolar and alveolar/bronchiolar have a more specific definition in classification of human primary lung tumors (Nikitin et al. 2004). This has at times resulted in some confusion, and some have recommended that these terms be abandoned in favor of classification and description based solely on the perceived histologic type (solid, papillary, mixed, etc.).

(Belinsky et al. 1992, 1991; Boorman 1985a; Boorman 1985b; Boorman 1985c; Breeze and Wheeldon 1977; Dixon et al. 1991; Dixon and Maronpot 1991; Dungworth, Ernst, et al. 1992; Dungworth, Hahn, et al. 1992; Dungworth et al. 2001; Foley et al. 1991; Gunning, et al. 1991; Gunning, Stoner, and Goldblatt 1991; Gunning, Goldblatt, and Stoner, 1992; Heath, Frith, and Wang 1982; Howroyd et al. 2009; Kristiansen et al. 1993; Malkinson 1989; Maronpot et al. 1991; Matsuzaki 1975; R. A. Miller and Boorman 1990; Mohr et al. 1990; 2006; 2006; Nikitin et al. 2004; Ohshima et al. 1985; Pour et al. 1976; Rehm et al. 1991; Rehm and Ward 1989; Rehm et al. 1991; Rehm, Ward, and Sass 1994; Rehm et al. 1988;

Reznik-Schüller and Reznik 1982; Rittinghausen et al. 1992; Rittinghausen et al. 1996a; Rittinghausen et al. 1996b; Schüller 1987; Schüller 1990; Singh et al. 1985; Thaete et al. 1987; Thaete and Malkinson 1990, 1991; Ward et al. 1983; Ward and Rehm 1990; Ward et al. 1985; Witschi 1985; Yamamoto et al. 1989)

Carcinoma, Acinar (Mice Only) (Figure 116): Terminal Bronchioles, Alveoli

Pathogenesis/cell of origin: Acinar carcinomas are believed to originate directly from bronchiolar epithelial (Clara) cells of the terminal airways by malignant transformation, or to arise from Clara cells populating alveolar walls. Some investigators believe that these tumors arise from parenchymal Clara cells that have migrated from bronchioli to the alveolar epithelium, whereas others consider them to arise from metaplasia of alveolar Type II cells.

Diagnostic features:

- Diffusely expansile with irregular margins or more circumscribed nodule
- Predominantly glandular/acinar pattern representing tumor cells that utilize existing alveolar walls
- Composed of cuboidal to columnar or pleomorphic cells without distinguishing features, or, more commonly, mixed with ciliated cells, or mucous cells
- Large portions or the entire tumor may show differentiation in favor of single cell type, for example, mucinous adenocarcinoma.
- Cells may show variable cytoplasmic eosinophilic globules.
- Lack of significant squamous cell metaplasia
- Neoplasms show clear features of malignancy such as penetration of basement membranes and tissue destruction.

Differential diagnoses:

- Carcinoma, bronchiolo-alveolar: Distinct nodular mass with cells forming papillary structures. Composed of cuboidal to columnar cells, lacking ciliated, mucous or squamous cells
- Carcinoma, adenosquamous or carcinoma, squamous cell: Tumor is composed in part (adenosquamous carcinoma) or entirely (squamous cell carcinoma) of malignant squamous cells.

Comment: Naturally occurring acinar carcinomas are extremely rare. They can be induced by intratracheal instillation of methylcholanthrene or cutaneous treatment with N-nitrosobis-(2-chloroethyl)urea. The latter does not cause the induction of solid/papillary neoplasms in strains of mice with low spontaneous lung tumor incidence. Cytoplasmic eosinophilic globules (eosinophilic intracytoplasmic proteinaceous accumulations) may be a prominent feature in the cells of acinar carcinomas and may also be found in nonneoplastic and hyperplastic cell pulmonary lesions. These globules are generally considered to represent dilated endoplasmic reticulum containing proteinaceous material.

(Dungworth et al. 2001; Rehm et al. 1991; Rittinghausen et al. 1996; Rittinghausen et al. 1996a; Rittinghausen et al. 1996b)

Carcinoma, Adenosquamous (Figure 117): Terminal Bronchioles, Alveoli

Synonym(s): Carcinoma, mucoepidermoid.

Pathogenesis/cell of origin: Rats: Bronchiolo-alveolar adenocarcinoma arising from alveolar Type II and/or Clara cells, presumably with clonal shifts to malignant squamous cell phenotype.

Mice: Adenosquamous carcinomas are believed to derive from acinar carcinomas, or possibly bronchiolo-alveolar carcinomas, with clonal shifts to malignant squamous cell phenotype.

Diagnostic features:

- Nodular, or diffusely expansile with irregular margins
- Composed of significant amounts of both adenocarcinomatous and malignant squamous cell components
- Squamous cells may show keratinization with formation of central keratin pearls or expansion of acini by desquamated keratinized cells.
- Squamous cell differentiation may also be recognized by the formation of polygonal cells with prominent intercellular bridges lacking keratinization. Cells may also be greatly enlarged with atypical nuclei.
- Neoplasms usually show clear indication of malignancy such as penetration of basement membranes and tissue destruction.

Differential diagnoses:

- Carcinoma, bronchiolo-alveolar or carcinoma, acinar: Bronchiolo-alveolar carcinoma or acinar carcinoma with squamous cell metaplasia have a large neoplastic glandular part with an insignificant portion of more regular benign looking squamous cells.
- Carcinoma, squamous cell: The neoplasm is composed entirely of malignant squamous cells, although there might be entrapped airspaces lined by hyperplastic bronchiolar or alveolar Type II epithelial cells.

Comment: The distinction between adenosquamous carcinoma and bronchiolo-alveolar carcinoma with squamous cell metaplasia sometimes is not clear cut.

Mice: Adenosquamous carcinomas most frequently derive from a squamous cell shift in acinar or bronchiolo-alveolar carcinomas. This is not usually seen in papillary neoplasms.

(Boorman, Morgan, and Uraih 1996; Dixon and Maronpot 1991; Dungworth et al. 1992; Dungworth et al. 2001; Mohr et al. 1990; Rehm et al. 1991; Rehm, Ward, and Sass 1994;

Rittinghausen et al. 1992; Rittinghausen et al. 1996a; Rittinghausen et al. 1996b)

Carcinoma, Squamous Cell (Figure 118): Terminal Bronchioles, Alveoli

Synonym(s): Carcinoma, epidermoid.

Modifier: Non-keratinizing; keratinizing.

Pathogenesis/cell of origin: Malignant transformation of squamous cell metaplasia of alveolar epithelium and/or Clara cells.

Diagnostic features:

- Growth pattern in cell clusters or irregular nests with central keratinization (keratin pearls), or without overt keratinization but forming distinct intercellular bridges
- Cellular debris and necrosis may be common as well as the presence of inflammatory cells, especially neutrophils.
- Cells have cytologic features of malignancy (atypia, disorganization, and increased mitotic rate). Local invasion is more common in keratinizing than in nonkeratinizing tumors.
- Cells may frequently be quite pleomorphic, including the formation of very large cells ("giant cells") 60 μm or more at the largest diameter.
- May invade the adjacent lung parenchyma, pleura, vessels, and/or bronchi
- Frequently, marked scirrhous response

Nonkeratinizing:

- Lack of overt keratinization, but cells characteristically display distinct intercellular bridges
- Cells usually pleomorphic and may be small with scant cytoplasm resembling basal cells of the upper respiratory tract or may be large and eosinophilic with abundant cytoplasm

Rats:

- Nodule or small mass composed of nonkeratinizing squamous cells with basaloid to slightly larger appearance. Preexisting alveolar architecture might still be evident. The cells have cytologic features of malignancy (atypia, disorganization, and increased mitotic figures), but stromal or vascular invasion is not always evident.

Keratinizing:

- Amount of keratin ranges from scarce to abundant

Rats:

- Squamous cell carcinomas with large central mass of keratin and necrotic tumor tissue appear to arise by malignant transformation in the walls of benign keratinizing cystic squamous cell tumors.

Differential diagnoses:

- Metaplasia, squamous cell: Preserves the normal pulmonary architecture. Small size. Tends to be multifocal. Low degree of cellular atypia
- Carcinoma, adenosquamous: Neoplasm composed of squamous as well as nonsquamous, glandular elements that both show evidence of malignancy
- Carcinoma, bronchiolo-alveolar or carcinoma, acinar: Composed of papillary strands or acinar structures lined by cuboidal or pleomorphic cells, with no or insignificant squamous cell metaplasia

Rats:

- Epithelioma, cystic, epithelioma, kpithelioma, nonkeratinizing: No signs of cytological (atypia, disorganization, mitoses) or behavioral (invasion) features of malignancy

Comment:

Rats:

- Metastases to or invasion into the mediastinum is quite common feature
- Distinction between benign squamous cell tumor and well-differentiated squamous cell carcinoma is sometimes arbitrary.

Mice:

- Spontaneously occurring squamous cell carcinomas are extremely rare in the lungs of mice, and benign proliferations of squamous cells (keratinizing cysts and keratinizing cystic epitheliomas), as they are described for rats, have not been reported to occur in mice.

(Boorman 1985a; Boorman 1985b; Boorman 1985c; Boorman, Morgan, and Uraih 1996 Dixon and Maronpot 1991; Dungworth, Ernst, et al. 1992; Dungworth, Hahn, et al. 1992; Dungworth et al. 2001; Faccini, Abbott, and Paulus 1990; Kuschner and Laskin 1970; Lijinsky and Reuber 1988; Maeda et al. 1986; Mohr et al. 2006, 1990; Rehm and Kelloff 1991; Rehm, et al. 1991; Rehm Ward, and Sass 1994; Rittinghausen et al. 1992; Rittinghausen et al. 1996a; Rittinghausen et al. 1996b; Schüller 1990; Schulte et al. 1994; Shabad and Pylev 1970; Yamamoto et al. 1989)

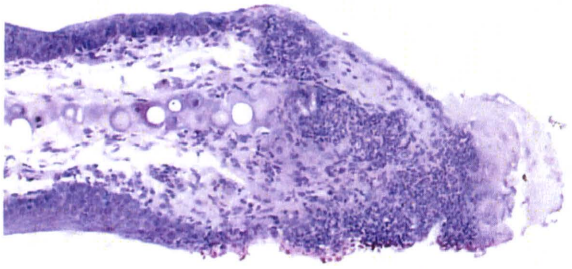

FIGURE 1.—Mouse. Perforation of nasal septum.

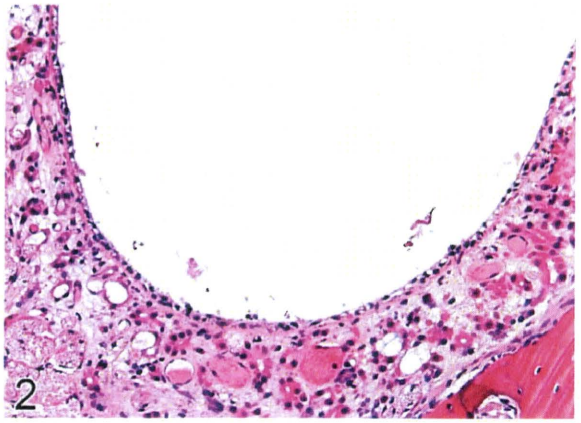

FIGURE 2.—Rat. Atrophy and necrosis, nasal septum.

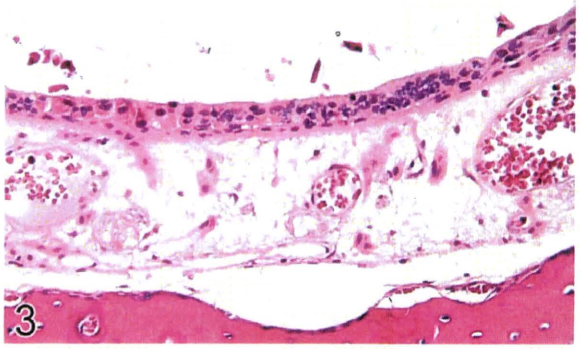

FIGURE 3.—Rat. Axonal and olfactory epithelium, atrophy.

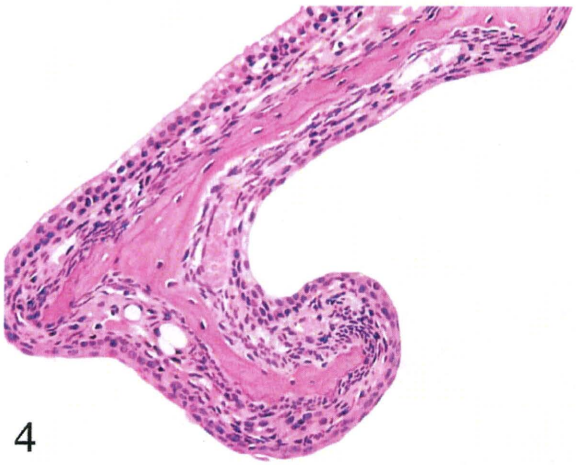

FIGURE 4.—Mouse. Nasoturbinate atrophy, acute inflammation, and squamous metaplasia.

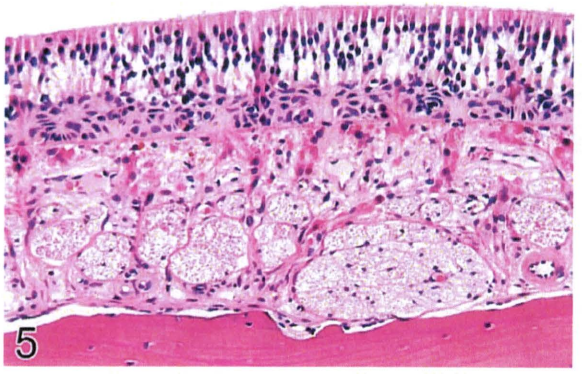

FIGURE 5.—Rat. Olfactory epithelial degeneration and basal cell hyperplasia.

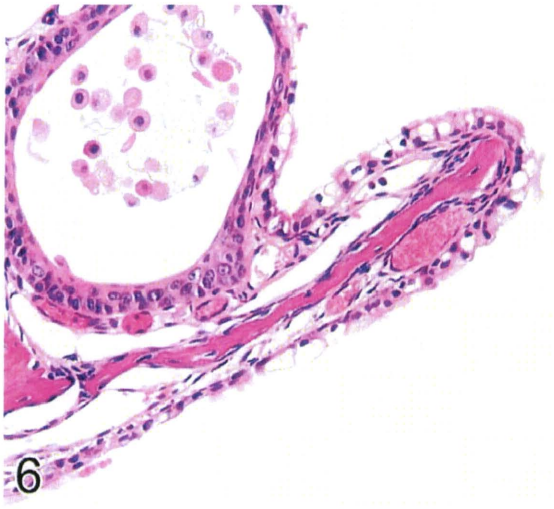

FIGURE 6.—Mouse. Cytoplasmic vacuolation of respiratory epithelium, nasal turbinate.