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V. Respiratory Tract

By far and away the most important pulmonary diseases in man are related to the smoking of tobacco. However, occupational lung diseases caused by inhalation of industrial chemicals, particulate matter and antigens are also important causes of morbidity and mortality. For this reason, considerable effort has been directed to the examination of airborne pollutants over recent years, including study of their effects in laboratory animals when administered by the inhalation route. Extensive study has shown that a complex array of defensive mechanisms protects the lung against the adverse effects of airborne substances and pathogenic organisms. Aerodynamic factors prevent access of particles larger than 10 µm diameter for these are deposited on the walls of the nasal passages. Particles measuring between 2 and 10 µm diameter tend to be trapped by the mucus-covered ciliated epithelium lining the bronchial tree and removed by mucociliary transport aided by the cough reflex. Smaller particles may reach the alveoli where they are ingested and transported by pulmonary macrophages (Murphy and Florman, 1983).

In contrast to the adverse pulmonary effects of cigarette smoke and industrial pollutants, therapeutic agents remain a relatively minor cause of pulmonary toxicity in man although actual incidence is difficult to ascertain. However, drug-induced pulmonary disease appears to be an increasingly frequent clinical problem and the drugs associated with parenchymal pulmonary injury in humans continue to increase (Hollinger, 1993). Drug-induced toxicity usually occurs after exposure of lung tissue via the circulation to either parent drug or metabolite.

The way in which drugs can affect the respiratory system in man have been summarised by Grant (1979). Hollinger (1993) has reviewed the specific drugs implicated. Through their specific pharmacological action drugs can produce excessive effects on bronchial calibre or pulmonary function. Drugs mediate allergic reactions in the bronchi or lungs. They may also produce a variety of obscure, diffuse pulmonary alveolar conditions including a pulmonary syndrome resembling systemic lupus erythematosus. As the respiratory tract is a major route by which microorganisms gain entry into the body, opportunistic pulmonary infections with
bacteria, viruses, fungi or protozoa are consequences of immunosuppression or broad-spectrum antibacterial therapy. As in other organs drugs that disturb coagulation may precipitate pulmonary thromboembolism or haemorrhage. Localised lung lesions also result from accidental, diagnostic or therapeutic inhalation of xenobiotics. Mucociliary clearance is also sensitive to therapeutic agents that affect the secretion of mucus and fluid, ciliary activity and transport (Sturgess, 1985). Treatment with antacids or histamine H₂ blockers can also increase the risk of pneumonia developing in patients in intensive care units through increasing gastric pH, which leads to an overgrowth of gram-negative bacteria in the stomach and retrograde pharyngeal colonisation (Craven et al., 1986).

In preclinical safety studies, pathology of the respiratory system can be the result of intercurrent disease or be induced by drugs administered systemically. Over recent years there has been increasing interest in the delivery of therapeutic agents by inhalation or intranasal routes (Ranney, 1986). Intranasal or inhalation modes of therapy pose particular challenges in terms of the novel formulations and the technologies required to administer drug as well as the potential impact of the different anatomical and physiological characteristics of the airways on drug toxicity, disposition and metabolism. The development of drugs to be administered by inhalation or intranasal routes is particularly difficult because of the perceived risks of high local drug concentration in respiratory tissues, alterations to drug disposition and a potentially vulnerable patient population, often with pulmonary disease (DeGeorge et al., 1998).

**Inhalation toxicology**

A complex science and technology has been developed to support the assessment of the adverse effects of inhaled substances in rodent and non-rodent species and the extrapolation of the experimental findings to man (Dorato, 1990; Dorato and Wolff, 1991). In order to administer xenobiotics, including drugs, by inhalation, it is necessary to generate aerosols (suspensions of particles in a gas) with a well-defined composition, particle size and shape. They must be delivered to the respiratory tract of laboratory animals in a way that parallels the likely human exposure. In case of therapeutic agents, this should avoid non-respiratory pathways through the skin and food.

When aerosols are inhaled, various fractions of the particles are deposited at different locations in the respiratory tract. Site of deposition depends primarily on particle size, but variability in the sites of deposition occurs among different laboratory animal species and man by virtue of the differences in the size and shape of the respiratory passages as well as breathing patterns (Sweeney and Brain, 1991). The relative distribution of monodisperse aerosols in man and rat were found to be comparable when particles of 1–3 µm diameter were inhaled, but different above this size range (Raabe, 1980).

The subsequent fate of inhaled particles depends not only on their size but also their shape, chemical nature, and solubility in body fluids. Soluble sub-
stances are absorbed into the blood stream and are removed by the pulmonary circulation. They may also undergo metabolism by enzymes present in the cell populations of the respiratory tract and reactive metabolites may cause local pulmonary damage. Insoluble, inert particles are removed primarily by the mucociliary transport system of the trachea and bronchi or through phagocytosis by macrophages. Overload of the lung by even relatively inert, non-fibrous particles such as titanium dioxide or carbon black may impair alveolar macrophage-mediated particle clearance (Warheit et al., 1995). This may lead in turn to accumulation of dusts over time with eventual fibrotic and tumorigenic responses.

A key component of the evaluation of the effects of inhaled substances is careful morphological assessment of the fixed tissues. Measurements of respiration rate, tidal volume, airway resistance, pulmonary gas exchange and the disposition of the inhaled substances also have a critical place in the evaluation of chemically induced lung damage in laboratory animals (Nemery et al., 1987). Several variables in the respiratory pattern of rodents can be measured simultaneously with whole body plethysmography using only modest restraint (Coggins et al., 1981; Costa, 1985).

NOSE, NASAL SINUSES, NASOPHARYNX AND PHARYNX

The nasal chambers are the structures which are first to be subjected to the effects of inhaled substances, whether microorganisms or chemical substances. Although these chambers are not usually examined in great detail in conventional toxicity studies in which substances are administered orally or by parenteral routes, they are carefully examined histologically when drugs are administered by inhalation.

Study of nasopharyngeal silicone rubber casts has shown considerable species differences in the anatomy of this part of the airway (Patra, 1986; Harkema, 1990, 1991; Resnik, 1990). Relative to total nasal length, the nasopharynx is longest in rat and shortest in man with the dog in an intermediate position. Maxilloturbinates are relatively simple structures in man and non-human primates but highly complex in dogs and rodents. As a consequence, regional nasal airflow and disposition patterns vary considerably and this influences the distribution of lesions produced by inhaled xenobiotics in the nasal cavity (Morgan and Monticello, 1990).

The anterior nares are lined by stratified squamous epithelium. In other zones the sinuses are covered either by respiratory or olfactory epithelium with a zone of transitional epithelium at the junction between the two types. Respiratory epithelium is similar to that found elsewhere in the respiratory passages being composed of ciliated cells, serous and mucous cells, brush cells, intermediate cells and progenitor basal cells. It represents a cellular system engaged in mucociliary clearance carrying surface secretions to the nasopharynx to be cleared by swallowing (Proctor, 1977). Although this epithelium is similar to that lining the other large airways, key differences are the particularly rich complement of secretory cells.
and the complex vasculature of the nose which can modulate capillary, arterial and venous blood flow through the mucosa (Proctor, 1977). Mucins may be particularly important. It has been postulated that they not only have a physical protective function but also possess antioxidant properties by virtue of the scavenging properties of their high proportion or sugar groups (Cross et al., 1984).

The proportion of the nose lined by olfactory mucosa is variable between species being disposed over a much larger area in dogs and rodents than in primates (Harkema, 1991). However, it is structurally similar in man and rodents. It is located in more dorsal or posterior regions of the nasal passages out of the direct line of airflow during normal respiration. Olfactory mucosa is a pseudostratified columnar epithelium composed of basal cells, sustentacular cells and sensory cells with mucus-secreting Bowman’s glands situated in the lamina propria. Basal cells are composed of two distinct types, light and dark cells. The light type represents the primitive, stem cell population. Sustentacular or supporting cells are non-ciliated, columnar cells possessing microvilli that extend into the overlying layer of mucus.

Cell bodies of olfactory sensory neurons are situated in the middle layer of the epithelium between sustentacular and basal cells. Their dendritic processes extend above the epithelial surface to end in a ciliated expansion referred to as the olfactory vesicle that is believed to be the receptor of odour perception. Olfactory axons extend from the cell body, penetrate the basement membrane in bundles to become surrounded by Schwann cells and eventually join with the olfactory bulb.

The olfactory system is of importance in toxicology for it can be selectively damaged by xenobiotics, including therapeutic agents, presumably as a result of its high metabolising capacity. The superficial location of neural cells in the olfactory epithelium also provides a model system for the study of the effects of xenobiotics on neural cells.

Submucosal mucous glands have been well characterised in both the rat and hamster where they are divided into lateral nasal glands and maxillary recess glands. These are both situated in the posterior parts of the nasal cavity and composed of mucus-secreting cells (Vidic and Greditzer, 1971; Adams, 1982). Similar glands have also been characterised in the nasal cavity of the dog (Adams et al., 1981).

Immunocytochemical study using antisera raised against the major isoenzymes of rat hepatic microsomal cytochromes P450 induced by β-naphthoflavone, 3-methylcholanthrene, phenobarbitone and pregnenolone-16α-carbonitrile as well as NADPH-cytochrome P450 reductase, epoxide hydrolase and glutathione S-transferases B, C and E has shown the presence of immune-reactive enzymes in the rat nasal mucosal cells (Baron et al., 1988). This suggests that the nasal mucosa not only has a capacity for metabolising and activating xenobiotics by oxidation, but also for hydration and inactivation of potentially toxic epoxides and conjugating electrophilic, reactive metabolites with reduced glutathione. In addition, it has been shown that the distribution of immune-reactive enzymes is different in olfactory and respiratory mucosa and the differences correlate with activity shown by enzyme histochemical methods (Bogdanffy, 1990). Xenobiot-
ics can be metabolised within both olfactory and respiratory mucosa, but the olfactory regions appear to possess greatest capability for oxidative metabolism. Consequently, regional differences in nasal toxicity and tumour formation from inhaled materials may not only be a response to different water solubility and deposition patterns but also differences in the formation of reactive metabolites (Bogdanffy, 1990). Another feature of this metabolising activity is that it can be induced by systemically administered xenobiotics. This occurs in a way that can alter the distribution of enzyme activity in the nasal mucosa (Baron et al., 1988).

In rodents, the relatively small size of the nose and nasal sinuses facilitates histological examination. Usually this area is sectioned transversely into several standardised blocks following decalcification (Young, 1981). There have been a number of detailed publications describing the histological preparation and assessment of pathology of the rodent nasal cavity (Tyler et al., 1985; Uraih and Maronpot, 1990). Morgan (1991) has described the approaches that can be adopted for the identification and recording of lesions in toxicity studies. In larger species, particularly dogs and primates sectioning and blocking is more complex. Although dissection is required, a similar procedure following decalcification can be adopted (Levinski et al., 1981). Examination of cytokeratin expression in the respiratory mucosa can also be used as a marker of epithelial differentiation in the respiratory tract. Schlage et al. (1998) have published a very detailed account of cytokeratin patterns in rat nasal mucosa for this purpose.

Like many other tissues exposed to external environmental agents, the nasal mucosa possesses aggregates of lymphoid tissue in the underlying lamina propria. In rats these areas, characterised by follicles containing both T- and B-cell areas are located in the ventral aspects of the lateral walls of the nasal airways at the opening of the nasopharyngeal duct (Spit et al., 1989; Harkema, 1991). Like the gut-associated lymphoid tissue, these nasal follicles have been shown in the rat to be covered by specialised epithelium with islands of cells with microvilli, so called M or membranous cells (Spit et al., 1989).

A test system that relates to the innervation of the nasal mucosa is that proposed by Alarie (1966). This enables the detection of airborne sensory irritants and the prediction acceptable levels of exposure to the upper respiratory tract in man. The trigeminal nerve endings in the nasal mucosa of mice mediate the response to sensory irritants and this can be measured by a decrease in respiratory rate. It has been shown that a good correlation exists between the decrease in respiration rate in mice exposed to airborne chemicals and the nasal irritancy potential of the chemicals in man (Alarie, 1981).

### Inflammation, ulceration, (rhinitis, sinusitis)

**Microbial pathogens**

Infectious agents cause inflammation in the nose and nasal sinuses and this may be associated with inflammation in the conjunctiva, middle ear and oral cavity.
Murine pathogens may cause alterations in the respiratory tract that can confound the assessment of changes induced by xenobiotics (Everitt and Richter, 1990). In rats, microbiological agents implicated in the development of rhinitis and sinusitis include *Corynebacterium kutscheri* (pseudotuberculosis), *Streptococcus pneumoniae*, *Pasteurella pneumotropica*, *Klebsiella pneumoniae*, *Mycoplasma pulmonis* and the sialodacryoadenitis virus or rat corona virus (Greaves and Faccini, 1992). Rats infected with the sialodacryoadenitis virus, show inflammation and necrosis of the upper respiratory epithelium as well as salivary and lachrymal glands. The Sendai virus, a paramyxovirus, also has marked tropism for the respiratory tract, including the nasal cavity and is associated with systemic effects that can compromise studies in laboratory rodents. Occasionally, fungal infections of the airways with *Aspergillus fumigatus* are reported (Everitt and Richter, 1990).

A variable, which has been shown to influence the severity of the rhinitis produced by *Mycoplasma pulmonis*, is the strain of rat. Following housing of LEW and F344 strains together to eliminate microbial and environmental differences, Davis and Cassell (1982) showed that the LEW strain developed a more severe rhinitis following inoculation with *Mycoplasma pulmonis* than F344 rats, although the reason for the difference was unclear.

Rat exposed to ammonia, a common pollutant of the air in laboratory animal cages have also been shown to develop lesions of the dorsal meatus, dorsal nasal septum and prominence of the turbinates. These lesions are characterised histologically by swelling or mild degeneration of the epithelium (Pinson et al., 1986). It appears that ammonia exposure can potentiate the acute inflammatory response of the nasal cavity to microbiological pathogens (Pinson et al., 1986).

A microorganism reported in the nasal cavity of rhesus monkeys employed in inhalation studies is the nematode of the genus *Anatrichosoma* (Klonne et al., 1987). Sections of this nematode are found in the squamous epithelium of the nasal vestibule and are associated with acanthosis and hyperkeratosis of the epithelium and a multifocal or diffuse granulomatous inflammation in the submucosa.

**Xenobiotics**

Administration of toxic or irritant substances to laboratory animals by the inhalation route produces degenerative, inflammatory and reactive changes in the nasal mucosa with a range of histological features similar to those found in mucosal surfaces damaged by other exogenous agents.

An example of the type and distribution of the degenerative and inflammatory conditions which can be induced by inhaled irritants is provided by the study in which Swiss-Webster mice were given various irritants by inhalation for periods of 6 hours/day for 5 days at concentrations which produced a 50% decrease in respiratory rate (Alaric test). Although the degree of histological changes varied with different agents, the changes were broadly similar in type and distribution (Buckley et al., 1984).
Most agents examined produced little or no alteration in the squamous mucosa lining the anterior part of the noses apart from some mild increase in thickness of the squamous epithelium. Principle sites of damage were shown to be the anterior respiratory epithelium adjacent to the vestibule and the olfactory epithelium of the dorsal meatus. There was a distinct decrease in severity in posterior regions.

Histologically, the lesions in respiratory epithelium ranged from mild loss of cilia and small areas of epithelial exfoliation to frank erosion, ulceration and necrosis of the epithelium and underlying tissues including bone. Variable polymorphonuclear cell infiltration was also found. In some cases, early squamous metaplasia developed on the free margins or the naso-maxillo-turbinates. Changes to the olfactory epithelium varied from focal to extensive loss of sensory cells associated with damage to sustentacular cells. In severe cases, complete loss of olfactory epithelium occurred.

Although the degree of histological change was shown to vary with different agents, lesions induced by the more water-soluble chemicals tended to remain localised in the anterior part of the nasal cavity whereas agents with relatively low water solubility produced lung lesions in addition. It was suggested that these findings demonstrated the powerful ‘scrubbing’ action of the nasal cavity for water soluble, airborne xenobiotics (Buckley et al., 1984).

Similar inflammatory alterations can be induced in the nasal cavity of rodents treated with therapeutic agents by inhalation. However, the precise relevance of such changes for human therapy by the inhalation route are sometimes questionable when the nasal damage is limited to high doses and it is not associated with alterations in other parts of the respiratory tract. In the case of tulobuterol, a \( \beta_2 \) adrenergic receptor agonist, it was argued that the nasal inflammation induced in rats in a 1 month inhalation toxicity study was the result of a particularly high exposure of the nasal epithelium to drug, not representative of the likely human exposure to tulobuterol by inhalation, where little or no nasal exposure would occur (Dudley et al., 1989). Walsh and Courtney (1998) described nasal epithelial degeneration and necrosis in both rats and dogs treated with a candidate anti-inflammatory drug by the intranasal route. This agent affected olfactory epithelium more than respiratory mucosa suggesting that metabolism was important in the generation of this toxicity.

Two further instructive examples of drugs that cause toxicity to the rat olfactory mucosa are provided by a novel type IV phosphodiesterase inhibitor, RP 73401 and methimazole, a thioureylene antithyroid drug used in clinical practice.

RP 73401 [3-cyclopentoxyloxy)-N-(3,5-dichloro-4-pyridy)-4-methoxybenzamide], which was being developed for the treatment of asthma and rheumatoid arthritis, produced degeneration of the olfactory epithelium in rats but neither dogs nor mice after single and repeated oral doses and by inhalation (Pino et al., 1999). Histologically, the olfactory epithelium showed necrosis of the superficial epithelial layers including the sustentacular and sensory cells with sparing of the basal cell layer. There was also damage to Bowman's glands. The development of proliferative lesions and ultimately tumours of principally neuroectodermal
origin followed chronic treatment. As RP 73401 was highly metabolised and the nasal lesions could be inhibited by treatment of rats with metyrapone, a non-specific inhibitor of cytochromes P450, it was postulated that the changes were the result of P450-mediated activation in the olfactory tissues, not linked to its pharmacological phosphodiesterase activity (Pino et al., 1999).

Methimazole, in contrast to RP 73401, is used in clinical practice where oral doses of 0.2–2 mg/kg/day are employed. Administration of methimazole at relatively high doses to Long–Evans rats by single oral (50 mg/kg) or intraperitoneal (25 mg/kg) routes also produced complete damage to the sustentacular and sensory cells with sparing of the basal cells and basement membrane (Genter et al., 1995). Bowman’s glands were also involved. Methimazole is metabolised by the flavin-containing monoxygenase system particularly as it is also employed as a model substrate for this enzyme in vitro. Genter et al. (1995) demonstrated the presence of flavin-containing monoxygenase isoforms in olfactory microsomes of Long–Evans rats and postulated that reactive intermediates were responsible for the nasal toxicity.

The risk of damage to human olfactory cells from agents with these effects in rat nasal mucosa often remains uncertain because an understanding of relative exposure and metabolism in different species and a better understanding of the metabolic potential of human olfactory mucosa is required.

Inclusions of the nasal mucosa

A particular response of the rodent nasal mucosa to some irritant substances, including pharmaceutical agents, is the formation of rounded eosinophilic inclusions in the cytoplasm of sustentacular cells of the olfactory epithelium and to a lesser extent in respiratory and glandular epithelial cells (Buckley et al., 1985; Gopinath et al., 1987). These inclusions are PAS-negative and ultrastructural examination shows that they are membrane-bound, ellipsoid bodies containing homogenous electron dense matrix. Their significance remains uncertain.

Proliferative lesions of the nasal mucosa

A standard classification for the variety of proliferative but non-neoplastic changes found in the rat nasal cavity representing largely adaptive responses to injury has been defined in the Society of Toxicologic Pathologists classification by Schwartz et al. (1994). Similarly, atypical epithelial lesions and neoplasms have also been included in this standardised classification. The classification of the International Agency for Research on Cancer provides a similar perspective (Mohr, 1992).

These lesions may be occasionally seen in untreated rodents in carcinogenicity studies but are much more commonly induced by administration of xenobiotics in inhalation carcinogenicity studies. Spontaneous nasal tumours are uncommon but most often squamous in type in rats whereas in mice spontaneous squamous
tumours are extremely rare and haemangiomas and respiratory adenomas pre-
dominate (Brown et al., 1991; Haseman et al., 1998). The generally agreed cat-
egories are described below:

**Mucous (goblet) cell hypertrophy and hyperplasia**

These changes affect the nasal respiratory epithelium and are characterised by
the presence of enlarged mucus-filled goblet cells some of which form clusters
suggestive on intraepithelial glands.

**Squamous cell hyperplasia**

This is seen in the stratified squamous epithelium of the nares and is character-
ised by a focal increase in the number of cell layers. Cells may show atypia with
irregular enlarged, pleomorphic nuclei and nucleoli.

**Squamous metaplasia**

Squamous metaplasia occurs to respiratory epithelium under conditions of
chronic damage. It is characterised histologically by the presence of three or
more layers of epithelial cells with eosinophilic cytoplasm and clear cell bounda-
ries whereas advanced lesions show typical keratinization and formation of inter-
cellular bridges. Cellular atypia may also be seen and should be characterised
when found.

**Respiratory epithelial metaplasia (of the olfactory epithelium)**

This represents atrophy and degeneration of the olfactory epithelium with loss
of sensory cells and in advanced cases loss of sustentacular cells with replace-
ment by ciliated and non-ciliated respiratory epithelium. It may be seen as a
spontaneous focal lesion in aged rats.

**Epithelial hyperplasia with cellular atypia (atypical hyperplasia, basal cell
hyperplasia, dysplasia)**

The term epithelial hyperplasia with cellular atypia is used to embrace prolif-
erative lesions in the respiratory and olfactory mucosa in the nasal cavity in
which there is varying degrees of altered differentiation and atypia. There is per-
turbation of the growth pattern of the epithelium such that the changes are not
those found in the normal regenerative response to transient mucosal damage.

**Adenoma (polypoid or villous adenoma, adenomatous or villous polyp**

Adenomas usually develop in the anterior part of the nasal cavity and are usually
exophytic lesions that develop from respiratory epithelium or nasal glands. Ad-
enomas of respiratory epithelium may be papillary in form but are by definition well circumscribed with minimal cellular pleomorphism and atypia. They may very occasionally occur spontaneously in aged rats (Brown et al., 1991). Adenomas of nasal glands usually show an acinar pattern.

**Squamous cell papilloma**

These develop in the squamous epithelium of the nares or in areas of squamous metaplasia in respiratory or olfactory epithelium. They are exophytic lesions with limited connective tissue stroma. They may develop spontaneously in aged rats (Hayashi et al., 1998).

**Carcinoma**

Both squamous cell carcinoma and adenocarcinoma develop in the nasal mucosa. Histologically, they have similar characteristics to those in other epithelial tissues. They are rare spontaneous lesions in aged laboratory rodents but may be induced by xenobiotics administered by inhalation, orally or by the parenteral route. Squamous carcinomas have been reported to develop in a small number of untreated Fischer 344 rats used in carcinogenicity studies in association with point mutations in the c-H-ras and c-K-ras gene (Hayashi et al., 1998).

**Olfactory neuroblastoma (ethesioneuroblastoma, olfactory neuroepithelioma, olfactory neuroepithelial carcinoma)**

These neoplasms show olfactory differentiation and arise from olfactory epithelium. They do not seem to occur as spontaneous lesions in rats or mice (Brown et al., 1991; Schwartz et al., 1994). Cells are arranged in lobules or in solid sheets with scanty stroma. Cells are relatively uniform with scanty cytoplasm with round or oval hyperchromatic nuclei. True rosettes with lumens or pseudorosettes are also seen. Poorly differentiated tumours of this type may require ultrastructural study for diagnosis. Olfactory neuroblastomas typically show the presence of electron-dense neurosecretory granules, neurofilaments or axons. As there is no detailed understanding of the biological behaviour of these neoplasms in laboratory rodents, the generic term olfactory neuroblastoma is usually preferred. They are almost always invasive tumours (Brown et al., 1991).

Olfactory carcinomas forming glands, follicles and rosettes have been occasionally reported in aged Syrian hamsters (Pour et al., 1976, 1979).

**Mesenchymal neoplasms**

These may be seen in the nasal cavity, particularly after exposure to potent carcinogens. Their histological features are similar to those in the soft tissues and bone elsewhere in the body (see chapter I).
LARYNX AND TRACHEA

The mucosa lining the larynx and trachea becomes involved as part of an upper or lower respiratory tract infection. For instance, in rats, an acute laryngitis or tracheitis has been shown to accompany experimental infection with *Mycoplasma pulmonis* and the sialodycroadenitis virus (Davis and Cassell, 1982; Wojcinski and Percy, 1986). A spontaneous degenerative condition of tracheal and laryngeal cartilage associated with granulomas has been reported in Fischer 344 rats (Germann et al., 1995). The cause of this condition is unknown but it increases in severity and incidence with advancing age although it is seen in rats as young as 6 weeks of age.

The larynx of rodents is also susceptible to the effect of inhaled substances, notably tobacco smoke but also pharmaceutical agents and propellants (Coggins et al., 1980; Gopinath et al., 1987; Sagartz et al., 1992). In view of the localised nature of induced lesions in the larynx, standardised histological sectioning techniques have been proposed for rats, mice and hamsters using anatomical landmarks (Sagartz et al., 1992; Renne et al., 1992; Renne et al., 1993).

The target site is located on the ventral floor of the larynx near the base of the epiglottis cranial to the ventral laryngeal diverticulum. Lesions tend to occur in the ventrolateral region, which is covered by respiratory epithelium and the inner aspect of the arytenoid processes which is lined by squamous mucosa. The larynx responds to inhaled irritants by inflammatory, degenerative and regenerative changes in a manner similar to other regions of the respiratory tract. These include disruption of the epithelial cells, inflammatory cell exudation and infiltration, goblet cell hyperplasia and squamous metaplasia (Coggins et al., 1980). However, these changes are not specific to inhaled irritants but also occur as a response to natural respiratory tract pathogens in conventionally housed rats (Lewis, 1982).

The pseudostratified ciliated and non-ciliated mucosa of the trachea may also show pathological alterations in inhalations studies, although sites at the bifurcation (carina) are those often first affected. Consequently, the carina should be systematically included in examination of the respiratory tract for induced lesions (Schwartz et al., 1994).

Neoplasia

As in the nasal passages a range of proliferative lesions including squamous hyperplasia, mucous cell hyperplasia, as well as papilloma, carcinoma and mesenchymal tumours are occasionally reported in the airways in laboratory rodents.

BRONCHI AND LUNGS

In man and laboratory animals, the trachea terminates at the bifurcation giving rise to two main bronchi which serve left and right lungs. Depending on
species, the main bronchi subdivide into further branches which enter the different lobes. Various forms of branching are recognised.

Bronchi may arise as side-branches from a parent or stem bronchus (monopodial). The parent bronchus can divide into two equal daughter bronchus (dichotomous) or several daughter bronchi (polychotomous) (Yeh, 1979). Study of silicone rubber casts of the respiratory tract has shown that the bronchial trees of man and non-human primates are essentially dichotomous, in contrast to the monopodial pattern of rodents (Patra, 1986). The comparatively long trachea of the dog gives rise to dichotomous upper airways but monopodial branching develops peripherally within each lobe.

The size of the lungs is dependent on size and weight of the different species, although the dog has comparatively smaller body mass and higher airway dimensions compared to man (Patra, 1986). The number of lobes is species-dependent. The human lung possesses an upper and lower left lobe and an upper, middle and lower right lobe. This contrasts with the upper, middle and lower left lobes and a fourth, azygos right lobe in rhesus monkeys and baboons (Hartman and Straus, 1965; Patra, 1986). The dog has three lobes on both right and left sides. Rats, mice and hamsters show cranial, middle, caudal and postcaval right lobes with a single, left lobe in mice and rats and a superior and inferior lobe on the left side in hamsters.

Cell types lining the bronchi are generally similar between species although not all subtypes have been clearly determined in every species (reviewed by Sturgess, 1985). The majority of cells are the ciliated cells that are accompanied by variable but relatively smaller proportions of basal cells, intermediate cells, mucous or goblet cells, serous cells, neuroendocrine and brush cells. In addition, mucous cells line the adjacent bronchial glands (Dormans, 1983). Unlike the tracheal mucosa, which is pseudostratified, the mucosa of intrapulmonary bronchi is non-stratified.

Ciliated cells are tall, columnar cells attached to basal and intermediate cells by desmosomal junctions. Tight junctions exist between adjacent specialised cells at the apex. Each cell possesses 200 or more cilia that are engaged in mucociliary clearance (Serafini and Michaelson, 1977). The superficial cell surface also shows a pronounced glycocalyx. The cytoplasm of ciliated cells contains scattered profiles of rough endoplasmic reticulum, a supranuclear Golgi, numerous mitochondria particularly near the apex where a prominent cytoskeleton is also found. Mucous or goblet cells are typical mucus-secreting cells representing about 10% of the bronchial mucosa cell population in man but less than 1% in pathogen-free rats (Sturgess, 1985). The serous cell is a cylindrical or pyramidal cell containing small, round, closely packed serous granules (Dormans, 1983).

Basal cells are compact, pyramidal cells resting on the basement membrane, believed to be progenitor stem cells with the intermediate cells representing an intermediate stage of cell differentiation.

The mucus-secreting and ciliated cells form the cellular basis for the mucociliary clearance mechanism of the main conducting airways. The epithelium is covered by a mucous blanket that is fairly complete in man and rabbit but patchier
in the rat (Sturgess, 1985). The mucous layer is segregated into an upper layer or gel phase separated from epithelial cells by a serous layer or sol phase. The complex carbohydrates of the glyocalyx and secreted mucosubstances show species-related differences in their sugar residues, which can be demonstrated histochemically by the use of labelled-lectins (Geleff et al., 1986).

Mucociliary clearance mechanisms are sensitive to the effects of many therapeutic agents, particularly those that influence mucins, fluid or electrolyte balance and ciliary activity. Anaesthetic gases, barbiturates, narcotics and alcohol depress clearance function. By contrast, topical, oral or parenteral administration of β-adrenergic agonists, isoprenaline and adrenaline, produce a dose-dependent stimulation of mucociliary transport by an effect on ciliary beat frequency, probably mediated by increasing levels of cyclic adenosine monophosphate in ciliated cells rather than through vascular changes. Although basal mucociliary function is dependent of normal vagal tone, parasympathomimetic agents can affect mucociliary transport. Acetylcholine and cholinergic agents stimulate ciliary activity whereas anticholinergic drugs, atropine and hyoscine, inhibit ciliary activity and mucociliary transport. These substances may alter deposition on inhaled particles in the lung (Sturgess, 1985).

Clara cells or non-ciliated bronchiolar cells located in the bronchiolar epithelium, first described by Clara (1937), are small and cylindrical in shape with highly infolded nuclei, surface microvilli, well developed Golgi, abundant endoplasmic reticulum and characteristic oval, homogeneous electron-dense granules in the apical cytoplasm. In rat, rabbit and man the granules are PAS-positive, although they are usually considered PAS-negative in hamster and mouse (Dormans, 1983).

On the basis of ultrastructural cytochemistry using frozen thin sections, in order to preserve Clara cell granules, surfactant-type proteins with partial or complete identity to those secreted by type II pneumocytes have been identified in Clara cells of the rat (Walker et al., 1986). Immune-reactivity was greatest in the cytoplasmic granules but was also shown in the Golgi and endoplasmic reticulum suggesting that surfactant-type apoproteins are synthesised and secreted by Clara cells. Clara cells also are highly active metabolically and contain cytochrome P450-dependent enzymes.

In most laboratory rodents, the conducting airways terminate abruptly at the non-cartilaginous and non-alveolarized terminal bronchiole that opens directly into an alveolarized airway, the alveolar duct. This in turn communicates with the alveoli (Bal and Ghoshal, 1988). Squamous epithelial or type I cells form only about 10% of all lung cells but they line over 90% of the alveolar surface, by virtue of extremely long cytoplasmic extensions. The principle gas exchange takes place across this cell. In the rat, the typical thickness of this barrier is 20 nm for a cytoplasmic extension of a type I pneumocyte, 90 nm for basal lamina and 90 nm for an endothelial cell (Dormans, 1983). The type I cell contains juxtanuclear mitochondria and the long smooth cytoplasmic extensions contain many ribosomes and pinocytotic vesicles. The anatomical configuration and function of type I cells render them highly vulnerable to inhaled gases and particles.
The other main alveolar lining cell is the granular pneumocyte or type II cell which constitutes about 10% of all lung cells, but which covers only about 5% of the alveolar surface (Pinkerton et al., 1982). This cell does not possess the long cytoplasmic processes typical of type I cells and it shows many microvilli at its luminal surface. The cell cytoplasm contains rough endoplasmic reticulum, Golgi apparatus, some mitochondria and characteristic oval, osmiophilic lamellar inclusions. Surfactant, a microaggregate of phospholipid and protein which modifies alveolar surface tension at low inflation volumes, is secreted by type II alveolar cells. Ultrastructural immunocytochemistry has shown the presence of surfactant apoproteins in the synthetic organelles and in the lamellar bodies of these cells, in agreement with the concept that the surfactant apoproteins are synthesised in the rough endoplasmic reticulum, glycosylated in the Golgi and are stored in lamellar bodies (Walker et al., 1986).

Type II cells are more resistant to the damaging effects of xenobiotics and unlike type I cells, they retain the ability to undergo mitotic division. Following damage to type I cells, increased numbers of mitoses are evident in type II cells which results in the appearance of large undifferentiated epithelial cells which ultimately differentiate into type I and type II cells (Dormans, 1983).

The alveolar brush cell (type III cell) is a cell of disputed function. It is described in rodents and man and constitutes only 1–5% of all lung cells. It is a pyramidal cell located principally at the junction of septa with a microvillous brush border on the surface.

Neurosecretory cells (Kultschitsky or APUD cells) are located in the epithelial surface of the larynx, trachea bronchi, bronchioles and alveoli (Becker and Gazdar, 1985). These cells are oval or cuboidal with oval nuclei, argyrophilic cytoplasm which electron microscopic examination shows to contain dense core granules. The role of neuroendocrine cells in the lung is uncertain but immunocytochemical study has shown them to contain neurone-specific enolase and a variety of peptides similar to vasoactive intestinal peptide, bombesin, calcitonin, serotonin, leu-encephalin, β-endorphin and ACTH (Becker and Gazdar, 1985; Sturgess, 1985).

Cells lining the bronchi, bronchioles and alveolar walls are also capable of metabolising xenobiotics. Immunocytochemical study has shown the presence of immune-reactive cytochromes P450, NADPH cytochrome P450 reductase, epoxide hydroxylase and glutathione S-transferase in bronchial epithelial cells, ciliated bronchiolar cells, Clara cells, type II and possibly type I pneumocytes in the rat lung (Baron et al., 1988). Baron and colleagues showed that the different cell populations contained different amounts of enzymes, Clara cells containing the greatest concentrations of the phenobarbitone-inducible isoenzyme of cytochrome P450, NADPH-cytochrome P450 reductase and epoxide hydrolase. Studies of microsomal enzyme activity suggest that lung tissue contains fewer P450 isoenzymes than liver, principally forms 1A1, 2B1, 3A2 and 4B1 (Smith and Brain, 1991). Whereas P450 enzyme activity is highly concentrated in specific cell types, overall microsomal enzyme activity is low compared with liver on a per gram of microsomal protein (Smith and Brain, 1991). Pulmonary mi-
Crosomol mixed function oxidases can be induced to different degrees by cigarette smoke in various mouse strains (Abramson and Hutton, 1975).

Other important cells are the pulmonary alveolar macrophages and lymphocytes. Lymphocytes are found in the epithelium of the airways, in the interstitium of alveoli and as part of follicles in bronchial walls. Pulmonary macrophages form part of the specific human immune defence system of the lung, involving, as elsewhere in the body, antigen presentation. In the rat and mouse, distinctive populations of pulmonary macrophages have been described based on enzyme activities and reactivity to monoclonal antibodies against monocyte and macrophages surface determinates (van de Brugge-Gamelkoorn et al., 1985; Breel et al., 1988). Bronchus associated macrophages in rat and mouse have more acid phosphatase and less non-specific esterase activity than the populations found in the pulmonary alveoli and interstitial tissues.

An important aspect of the immune system is the bronchus-associated lymphoid tissue or BALT, which forms part of the mucosal lymphoid system found in other epithelia, notably the gastrointestinal tract. The morphology of BALT can be a useful guide to the nature and degree of immune stimulus reacting in the lung.

Structurally, the BALT is organised in a way that is characteristic of other peripheral lymphoid organs. Its organisation and function has been well studied in the laboratory rat where it closely resembles the BALT in other laboratory animal species including the mouse, rabbit, guinea pig as well as man (Bienenstock et al., 1973; van der Brugge-Gamelkoorn and Kraal, 1985; Breel et al., 1988). Its size and prominence is species and strain-dependent as well as a function of the degree of antigenic stimulus.

In the rat, the BALT is composed of lymphoid aggregates or follicles located mostly between a bronchus and artery with a zone of lymphocytes situated immediately under the bronchial epithelium. As in other peripheral lymphoid tissue, BALT is organised into B- and T-cell zones but in no predetermined manner. Immunocytochemical staining has shown that B- and T-lymphocyte zones differ in location from one aggregate to another.

In the rat BALT, it has been shown that there are about two T lymphocytes for every three B cells compared with a ratio of 2:5 in rat Peyer’s particles (van der Brugge-Gamelkoorn and Sminia, 1985). The ratios may be different in other species. In the rabbit, equal percentages of B and T cells have been found in both BALT and Peyers patches (Rudzik et al., 1975).

Quantitative observations of T-cell subsets using monoclonal antibody clones W3/13 (CD43), W3/25 (CD4) and MRC OX8 (CD8) have also shown that rat BALT contains twice as many T-helper as T-suppressor/cytotoxic lymphocytes (van der Brugge and Sminia, 1985). The T cells are confined to one or two discrete zones with a light scattering of T cells within the B-cell zones and immediately under the bronchial epithelium.

These numbers appear to correlate with the different numbers of B and T lymphocytes that enter these lymphoid aggregates through the high endothelial venules. Binding assays have shown that there are differences in the adherence
specificity of high endothelial venules in rat BALT compared with those in mesenteric lymphoid tissue (van der Brugge-Gamelkoorn and Kraal, 1985).

In common with lymph nodes, Ia-positive interdigitating cells are found in T-cell zones of BALT. In view of their expression of Ia antigen they can be demonstrated in the rat using the mouse monoclonal antibody MRC OX4 (van der Brugge-Gamelkoorn et al., 1985). In B-cell zones, interdigitating cells are also found and they can be demonstrated in rat BALT as in lymph nodes using mouse monoclonal MRC OX2 (van der Brugge-Gamelkoorn et al., 1985). The epithelium overlying BALT shows anatomical modifications. It is composed of ciliated and non-ciliated cells covered by microvilli.

The conventional, untreated laboratory rats, BALT shows little activity and germinal centres are usually absent, although BALT has been recently reported to be more prominent in some rat colonies in association with non-specific inflammatory lesions in lungs (Ewell and Mahler, 1997; Slaoui et al., 1998). BALT is also present in germ-free animals although it is less pronounced than in conventional rats (Bienenstock et al., 1973).

In the young Wistar rats studied by van der Brugge-Gamelkoorn et al. (1985, 1986) germinal centres were not seen in BALT in untreated animals but they developed following the administration of a single intratracheal dose of lipopolysaccharide, a T-cell-independent antigen. Single intratracheal doses of T-cell-dependent antigens such as horseradish peroxidase, bovine serum albumin and BCG, only produced minor morphological changes which included expansion of the zone of lymphocytes immediately under the epithelium and infiltration of the bronchial epithelium overlying BALT by lymphocytes. In addition, perivascular, peribronchial or alveolar infiltrates of small and large lymphocytes and macrophages were observed in the lungs of rats given BCG.

Immunocytochemical study of the rat BALT following intratracheal challenge with horseradish peroxidase showed that the majority of cells that infiltrated the bronchial epithelium were T helper (W3/25 or CD4 positive) lymphocytes (van der Brugge-Gamelkoorn et al., 1986). Furthermore, the Ia expression of the epithelial cells overlying the BALT was shown to increase, associated with electron microscopic evidence of an increase in the number and size of microvilli, a more pronounced glycocalyx and a decrease in number and size of cilia.

Immunocytochemical study of the BALT tissue in C57B1/6 mice using monoclonal antibodies to lymphoid and macrophage populations has demonstrated quite similar arrangements of cells to those in the rat with the majority of T cells belonging to the T helper (L3T4 or CD4 positive) class (Breel et al., 1988).

The pulmonary lymphatic system drains into mediastinal or cervical lymph nodes. Although among rat strains, differences in the location of lymph nodes and their drainage pattern occur, tracer studies in the Fischer 344 rat using colloidal carbon have shown that the lung lymphatics drain mainly into posterior mediastinal lymph nodes and those in the tracheal wall drains primarily to the internal jugular and posterior cervical nodes (Takahashi and Patrick, 1987).

The mechanisms by which lung damage occur in man are not always clear, particularly interstitial lung diseases, including those related to drug therapy.
Under certain circumstances, an initial insult may trigger a whole cascade of immune-mediated phenomena leading to chronic lung disease. Experimental evidence suggests that complement is an important source of mediator for the induction of acute pulmonary inflammation (Johnson et al., 1979). Stimulation of alveolar macrophages or neutrophils during phagocytosis or by chemotactic factors increases production of enzymes that have the potential to hydrolyse elastin and collagen. It is probable that the neutrophil is an important effector cell in IgG immune complex-induced lung injury. IgA immune complexes appear capable of producing lung injury in the rat by stimulating oxygen radical formation by macrophages (Johnson et al., 1988).

It has been shown that T lymphocytes are capable of inducing vascular injury in the lung (Anderson et al., 1988). T lymphocytes are important in the development of granulomatous pneumonitis induced in the mouse by BCG (Takizawa et al., 1986).

**Structural evaluation**

Although a variety of fixation, embedding and staining procedures are available for light and electron microscopic examination of lung tissue, there is no substitute for initial, careful visual inspection of the lungs at autopsy. Uneven collapse of lungs on opening the thoracic cavity, discoloration or alteration in texture of the pleural or cut surface, congestion and presence of fluid in the larger airways may indicate structural damage. In rodent lungs, small pulmonary adenomas may be detectable by inspection in good light.

Fresh lung weight is also a helpful measure in lung assessment, although passive vascular engorgement can significantly affect this value. Nevertheless, studies in the normal Fischer 344 rat have shown that after exsanguination, wet lung weights show a close relationship to body weight and that dry weight of lungs consistently represents about 20% of the wet weights regardless of age or body weight (Tillery and Lehnert, 1986). It has been suggested that an increase in wet weight over dry weight is a good index of pulmonary oedema. However as pulmonary transudate or exudate is protein rich, dry lung weight may also increase to a variable extent when there is an increase in pulmonary fluid (Nemery et al., 1987).

Various methods of fixation have been employed although simple immersion fixation in formalin for conventional light microscopy has the virtue of simplicity and it avoids the risk of translocation or removal of exudates from airways and alveoli. Mixtures of formaldehyde, paraformaldehyde and glutaraldehyde are used in initial fixation for electron microscopy (Tyler et al., 1985). Better appreciation of lung architecture is achieved by instillation of fixative via the trachea under an appropriate constant pressure or by perfusion fixation of the pulmonary arteries that is less liable to dislodge intra-alveolar exudate. However, even with care, significant artefact can result from airway instillation or vascular perfusion fixation (Michel, 1985).
The sampling procedure is an important aspect of histological examination of the bronchi and lungs, particularly from large laboratory animals. The extent of histological sectioning in conventional toxicity studies should be modulated to take account of lesions found by macroscopic examination, the type of study and the nature of the test substance. The bronchi should be carefully sampled to allow assessment of any alterations in bronchial epithelium.

Morphometric analysis represents a sensitive tool, which can be of great value in the evaluation of drug-induced lung changes, but it requires particularly rigorous sampling and evaluation procedures (Hyde et al., 1991). A tiered, multiple stage or cascade sampling technique is normally considered the most appropriate for morphometric studies (Barry and Crapo, 1985). This involves dividing the lung into a series of homogeneous compartments or strata from which randomly selected samples can be examined by appropriate light or electron microscopic techniques.

Immunocytochemistry and enzyme cytochemistry are helpful in the study of the heterogeneous cell population of the lung. Xenobiotic metabolising activity can be studied both by enzyme cytochemical methods as well as by immunocytochemical techniques using antisera specific for pulmonary monoxygenases and related enzymes. Important structural components, particularly collagen and laminin can be studied both at light and ultrastructural level with immunocytochemical methods (Gil and Martinez-Hernandez, 1984). Cytokeratin immunocytochemistry can also be used as a method for the characterisation of changes to epithelial cells (Schlage et al., 1998).

Endothelial cells of the Fischer 344 rat pulmonary vasculature may be demarcated immunocytochemically by some antisera to factor VIII-related antigens and this can be helpful in the study of changes in lung endothelium (Tillery and Lehnert, 1986), although this may not be a reliable stain for endothelial cells in all organs (see Integumentary System, Chapter I). Other useful antigens, which can be demonstrated in the lung, include surfactants, lysozyme, immunoglobulins and those of microorganisms that infect the lung (Linniola and Petrusz, 1984).

**Oedema**

Pulmonary oedema is a component of many inflammatory conditions of the lung including those induced by infections agents. However, the term oedema is reserved for a poorly cellular exudate characterised by the presence of pale, homogeneous eosinophilic material in the alveoli, sometimes associated with a similar exudate in the lung septae and perivascular connective tissue, rather than inflammatory exudate.

It occurs in a number of spontaneous conditions such as in congestive cardiac failure, accompanying metastatic pulmonary neoplasms or as an agonal change in association with pulmonary congestion and haemorrhage. Drugs may induce cardiogenic pulmonary oedema as a consequence of pulmonary hypertension or impaired ventricular contractility. Cardiogenic oedema is often associated with
vascular congestion and red blood cells and haemoglobin may leak into airspaces. This can give rise to the presence of haemoglobin crystals within the oedema fluid in formalin fixed tissue sections (Fig. 28).

Most importantly, pulmonary oedema may be a manifestation of acute lung injury. Inhalation or systemic administration of toxic chemicals may produce acute pulmonary oedema. Some substances such as phenylthiourea and α-naphthylthiourea produce massive pulmonary oedema in laboratory animals when administered orally, principally as a result of damage to the endothelium of pulmonary capillaries and venules (Keher and Kacew, 1985). Over 30 drugs have been reported to produce non-cardiogenic pulmonary oedema in humans either directly or through poorly understood immunogenic mechanisms (Hollinger, 1993).

Another form of pulmonary oedema involves the main airways. Precipitation of allergic reactions in sensitised airways of asthmatic individuals is believed to result from cross-linking of IgE and activation of mast cells that degranulate and release inflammatory mediators (Holgate et al., 1986). This has been shown to occur in the main airways of rats sensitised to ovalbumin and then challenged with ovalbumin by the intratracheal route (Lebargy et al., 1987). This treatment leads to rapid accumulation of bronchial exudate, degranulation of mast cells and the development of mucosal oedema, most marked immediately below the respiratory epithelium.

Fig. 28. Lung from a Wistar rat treated with high doses of a cardioactive drug that produced pulmonary oedema. This section shows the presence of haemoglobin crystals. Whilst these crystals may not form during life their presence suggests leakage of red blood cells into the air spaces. Illustration by courtesy of Dr Peter Wadsworth. (HE, ×80.)
Congestion and haemorrhage

Congestion and haemorrhage is a frequent finding in the lungs of laboratory animals where it is usually related to certain modes of death. It can be associated with administration of drugs and chemicals that have adverse effects on cardiac function or on the coagulation system. Administration of heparin to rats produces a characteristic extravasation of blood into the air spaces (Larsen et al., 1986).

Inflammation due to infections and infestations

Lower respiratory tract infection is generally not a major health hazard among laboratory animals but it is nevertheless an ever-present threat which can cause overt respiratory disease within a colony or develop following administration of xenobiotics. Subclinical pulmonary infections and infestations can also produce histological alterations in the bronchial airways or pulmonary parenchyma which mimic changes induced by inhaled irritants or systemically administered drugs (Elwell and Mahler, 1997; Slaoui et al., 1998). Furthermore, some respiratory pathogens alter immune defences and exacerbate the effects of inhaled substances (Jakab, 1981).

A range of bacterial and viral pathogens may produce inflammatory lung changes (Everitt and Richter, 1990). Typically, bacterial pathogens such as *Streptococcus pneumoniae* produce acute bronchitis associated with a variable degree of acute inflammation of the lung parenchyma (bronchopneumonia) or a confluent lobar pneumonia. Viral agents are generally associated with histological features of bronchiolitis and interstitial pneumonia, characterised by an increase in mononuclear cells in the respiratory bronchioles and alveolar septa. The histological features are variable for they depend on the particular pathogen, species and strain, immune status, presence or absence of secondary infection and the particular stage at which the infection is examined. Respiratory infections are frequently mixed. Changes due to secondary bacterial infection are frequently superimposed on those induced by viruses.

Sequential histopathological examination of the lungs of laboratory animals following inoculation with respiratory tract pathogens has been able to characterise the evolution of pathological changes produced by individual organisms. For instance, rats infected with *Mycoplasma pulmonis*, one of the more important intercurrent respiratory pathogens among laboratory rodents. Following inoculation, LEW and F344 rats were shown to develop an upper and lower respiratory tract inflammatory process. In the LEW strain this was characterised after 28 days by a variable acute inflammatory exudate in bronchi and bronchioles with focal bronchiectasis, inflammation and hyperplasia of the epithelium with a predominately macrophage infiltration of the alveoli and aveolar walls (Davis and Cassell, 1982). These changes were associated with marked hyperplasia of the bronchus-associated lymphoid tissue (BALT), which extended
further down the airways and blood vessels towards the periphery of the lungs. Although the lymphoid hyperplasia was also found in inoculated F344 rats, it was less marked and accompanied by little or no mucopurulent exudate or active inflammation of the bronchial walls.

Similar studies have been conducted in both rats and mice infected with another important respiratory pathogen of laboratory rodents, Sendai virus (para-influenza type 1). Sequential studies showed that the initial damage to bronchial and bronchiolar epithelium is associated with polymorphonuclear and lymphocytic inflammation (bronchiolitis). Immunocytochemical and ultrastructural studies revealed the presence of viral antigen in the mucosa (Jakab, 1981). Hyperplastic and multinucleated syncytiial epithelial cells develop in the hyperplastic terminal bronchiolar epithelium and the inflammatory process extended to involve peribronchial or peribronchiolar parenchyma with infiltration of alveolar walls by mononuclear cells, macrophages and neutrophils. A similar cell population accompanied by cell debris and oedema fluid develops in air spaces. Pulmonary arteries show only minor involvement with inflammatory cells and focal reactive hyperplasia of the endothelium. Immunocytochemistry and ultrastructural examination has suggested that virus replication take place in alveolar type I and type II epithelial cells and macrophages but not in endothelial or interstitial cells of the alveolar septae (Castleman et al., 1987). It was shown that repair occurs but there may be residual distortion of bronchiolar and alveolar walls by collagen. Increased tumour necrosis factor-α (TNF-α) expression has been shown to be an important regulatory factor in the development of Sendai virus-induced bronchiolar fibrosis in infected rats (Uhi et al., 1998). Hyperplastic cuboidal epithelium may line thickened alveolar septa and air spaces contain enlarged macrophages with pale vacuolated cytoplasm (Castleman, 1983).

The Corona virus, which causes sialodacryoadenitis in many rat colonies, also produces lower respiratory tract inflammation. This is characterised by acute bronchitis and bronchiolitis with focal extension into lung parenchyma. Thickened oedematous, hypercellular alveolar walls infiltrated by monocytic cells are found (Wojcinski and Percy, 1986). Immunocytochemistry has shown the presence of viral antigen in bronchial and bronchiolar epithelial cells. There is also peribronchial lymphocytic infiltration and increased prominence of BALT but ultimately complete resolution occurs.

Viruses remain a potential source of spontaneous respiratory disease in laboratory dogs. Canine adenovirus type 2, parainfluenza SV5, canine herpes virus, coronavirus and parvovirus have all been isolated from laboratory dogs developing respiratory disease (Binn et al., 1979). Castleman (1985) showed the evolution of histological changes and antigen localisation in the lungs of young beagle dogs experimentally inoculated with canine adenovirus type 2.

The syndrome of visceral larva migrans also incites focal inflammation, granulomas and fibrosis in the lungs of species such as dog and primate in which parasites are prevalent. The syndrome of visceral larva migrans is usually defined as that which results from the migration of nematode larvae into the viscera. It has been well described in the beagle dog lung where it results from the larvae
of toxocara species or metstrongyloid nematodes (Barron and Saunders, 1966; Hirth and Hottendorf, 1973). The precise identification of parasites is not always possible in tissue sections. Histological appearances of infested lungs are highly variable. Nematodes surrounded by granulomas and granulomatous inflammation, mostly in a subpleural location, may be visible in sections. In affected lungs there may be perivasculitis and active arteriolitis, bronchiolitis and peribronchialitis. Pleural involvement by the inflammatory process can be marked, particularly in regions overlying granulomas. Scarring develops and pleural and subpleural fibrosis frequently associated with epithelial hyperplasia and squamous metaplasia of the associated airways (Hirth and Hottendorf, 1973). The lesions may sufficiently marked to resemble those induced by high doses of anticancer drugs such as bleomycin (see below).

Pulmonary acariasis is a common infestation of many species of non-human primates caused by various species of the mite *Pneumonyssus*. Reproduction of the mites appears to take place in the terminal bronchioles. *Pneumonyssus simico*ola is the recognised form found in rhesus monkeys (Kim, 1988). Although it is most prevalent in wild caught primates, the disease is not easily eliminated during breeding in captivity (Joseph et al., 1984). As the mite can produce significant destructive pulmonary pathology and render animals susceptible to secondary pulmonary bacterial infections, it can disrupt or confound the interpretation of toxicity studies performed in primates. The lesions are located most frequently in cranial lobes and are characterised by the presence of bullae distending the pleural surface, parenchymal cysts, nodules and scar (Joseph et al., 1984; Kim, 1988). Histologically, there is a wide range of inflammatory activity. Fully developed lesions are characterised by granulomatous bronchiolitis and peribronchialitis with involvement of immediately adjacent alveoli. Cystic lesions involving the bronchiolar walls develop around the parasites giving rise to the appearance of walled-off cysts composed of highly cellular granulation tissue, associated with neutrophils, lymphocytes, macrophages, multinucleated giant cells and various pigments (see below). In less active lesions, dilated, cystic airways with walls composed of thick bands of smooth muscle and lined by squamous or cuboidal epithelium are found (Joseph et al., 1984).

*Pneumocystis carinii* is an important cause of pneumonia in patients with the acquired immunodeficiency syndrome (AIDS) as well as in other immunocompromised patients including those treated with immunosuppressive drugs (Walzer, 1986). The natural habitat of *Pneumocystis carinii* is pulmonary alveoli and it is widely encountered in the human population without being associated with overt disease. Both clinical and experimental evidence suggests that impaired cellular immunity is much more important as a predisposing factor than impaired humoral immunity (Walzer, 1986).

As in man, laboratory animals may have latent pneumocystis infection that becomes clinically evident following immunosuppression. It has been shown in the rat that chronic administration of various regimens of adenocorticosteroids, low protein diets, cyclophosphamide and other immunosuppressive drugs with concomitant antibiotic administration to prevent other infections gives rise to
typical pneumocystis pneumonia (Chandler et al., 1979). Rodents with genetically deficient cellular immunity also develop pneumocystis pneumonia. The importance of pneumocystis pneumonia in toxicology is that it can be considered as a sentinel of chronic immune depression.

In haematoxylin and eosin stained sections, pneumocystis pneumonia is characterised in both man and rodents by the presence of alveoli filled with foamy eosinophilic material containing a few macrophages and indistinct nuclei of pneumocystis (Fig. 29). Ovoid or crescent-shaped structures of the organisms become clearly visible with Gomori methenamine silver or toluidine blue stains.

Ultrastructural study of rats with pneumocystis pneumonia shows that trophozoites attach themselves most frequently to type I pneumocytes by altering their morphology to the contours of the pneumocytes rather than by a process of invasion (Long et al., 1986).

**Drug-induced inflammation**

Systemically administered therapeutic agents may produce histological changes within the lung parenchyma that mimic components of the normal response to respiratory pathogens. However, there is no sharp separation between agents
that produce pulmonary oedema and those that are associated with acute inflammatory changes and histological features overlap because an acute inflammatory process is often accompanied by exudate within airspaces.

An example of drug-induced pulmonary inflammation in laboratory animals and humans is reported following the administration of interleukin-2 (IL-2). IL-2 is a glycoprotein lymphokine, molecular weight 15,000, which is normally produced by activated T cells and mediates immunoregulatory responses. It has been produced in large quantities by recombinant DNA technology for use in tumour immunotherapy where high doses have been associated with a number of adverse effects, notably the 'vascular leak' syndrome. This syndrome is characterised clinically by pulmonary oedema, pleural effusions and ascites (Rosenberg et al., 1987).

The vascular leak syndrome has been reported in laboratory animals given high doses of this agent. Histological examination of the lungs of B6D2F1 mice developing this syndrome following administration of the IL-2 showed infiltration of the alveolar walls with large lymphocytes and intra-alveolar proteinaceous exudate containing large lymphocytes, macrophages and red blood cells (Anderson et al., 1988). Pulmonary venules and arterioles also showed the presence of lymphocytes attached to or lying beneath the endothelium, infiltrating vessel walls or in a perivascular location where they were accompanied by oedema fluid or red blood cells. Similar, but less severe changes have been demonstrated in rats given IL-2 (Anderson and Hayes, 1989). In addition, treated rats showed an infiltration of pulmonary vasculature with eosinophils probably secondary to an eosinopoietic cytokine produced by IL-2 stimulated lymphocytes.

Immunocytochemical evaluation of the lymphoid infiltrate in mice showed that most of the cells were Thy 1.2-positive (CD90) lymphocytes. Furthermore, co-administration of asialo GM1 (ganglioside) with interleukin-2 not only abrogated the clinical signs but also reduced the number of asialo GM1-positive lymphocytes in the tissue sections.

As lymphoid cells expressing Lyt-2 (CD8, suppressor/cytotoxic T cells) were unaffected by asialo GM1 treatment, it was postulated that the vascular leak syndrome (but not antitumour efficacy) in these mice was mediated by an endogenous subset of IL-2 stimulated lymphocytes or lymphokine-activated killer cells (Anderson et al., 1988). Corresponding changes were also observed in liver and lymphoid tissue (see Liver, Chapter VIII, and Haemopoietic and Lymphatic Systems, Chapter III). More recently, immunocytochemical and detailed electron microscopic studies in rats have supported the concept that IL-2 induces cytotoxic vascular damage that is mediated both directly by lymphokine-activated killer cells and cytotoxic T lymphocytes with secondary release of inflammatory cytokines (Zhang et al., 1995).

As in man, severe chronic pulmonary inflammatory disease in laboratory animals may compromise pulmonary function and lead to secondary alterations in other organs. Although the mechanisms were not explored in detail, a diffuse interstitial pulmonary inflammatory process with lung haemorrhage was induced in rats treated for 2 years with prizidilol (SK&F 92657-A2), an antihypertensive
agent with both vasodilator and β-adrenoceptor blocking properties (Sutton et al., 1986). Affected animals developed dyspnoea associated with reduction in lung volume, deformity of the thoracic spinal column and marked cardiac hypertrophy.

**Granuloma, granulomatous inflammation**

Inflammation with a granulomatous component develops in the lungs of laboratory animals under a variety of different circumstances, which have been alluded to above. Granulomas may be found without obvious causation but common cause in rodents is the response to accidentally inhaled foreign bodies. Lipid granulomas with cholesterol clefts and fibrosis develop focally in response to lipid released from foamy macrophages that accumulate in rats with increasing age or in drug-induced phospholipidosis. As dogs and primates are more liable to be infested by parasites, granulomatous inflammation in response to pulmonary larvae is more common in these species.

Another form of granulomatous pulmonary inflammation results from aspiration of stomach contents or food particles (aspiration pneumonia). This is sporadically observed in aged rats where it is associated with general ill-health, particularly resulting from pressure effects of large pituitary adenomas and subsequent disturbance of pharyngeal or laryngeal reflex mechanisms (Dixon and Jure, 1988). Histologically, the lungs show peribronchial and peribronchiolar granulomatous inflammation with macrophages and foreign body cells associated with fragments of refractile vegetable matter. The associated bronchial mucosa may also show reactive changes including goblet cell hyperplasia in long-standing cases.

Pulmonary tuberculosis represents a potential problem among non-human primate colonies in view of its insidious onset and its liability for transmission from monkeys to man (Wolf et al., 1988). Pathological findings are similar to those so well known in the human disease. The disease is characterised by the presence of granulomas in lung parenchyma and lymph nodes. In florid cases there may be caseation surrounded by epithelioid and multinucleated giant cells and variable numbers of lymphocytes, plasma cells and fibroblasts. Diffuse granulomatous pneumonia as a result of tuberculosis is also reported in non-human primates (Wolf et al., 1988).

Granulomatous pneumonitis is produced in laboratory animals by the intravenous injection of BCG. Twenty-eight days following intravenous injection of BCG, the lungs of C57B1/6 mice contained numerous granulomas composed of histiocytes and round cells which were surrounded by alveoli with thickened walls and associated with mild interstitial pneumonitis (Takizawa, 1986). These histological changes were associated with an increase in the number of Thy 1.2-positive (CD90) cells, especially Lyt-1 (CD5) positive lymphocytes. The histological changes were abrogated by treatment with cyclosporin A, suggesting an important role for CD5-positive lymphocytes in the development of the granulomas.
Discrete granulomas occur in the lungs of experimental animals in response to intracheal or intravenous injection of certain relatively insoluble substances. Intracheal administration of insoluble polymerised dextran and latex microparticles to mice showed that the morphology and the systemic effects of granulomas depended on the nature of the injected substances. Large granulomas rapidly developed in the pulmonary parenchyma around dextran particles that subsequently regressed quite quickly, whereas latex particles produced small, discrete stable granulomas (Allred et al., 1985). Although both forms of granulomas were of foreign body or non-immunological in type, those produced by dextran but not latex beads, were associated with anergy-like immunosuppression probably caused by release of soluble factors from the granulomas.

It has been reported that granuloma formation after instillation of sephadex beads is associated with increases in the interleukin-1 (IL-1) like activity in the lung (Kasahara et al., 1989). Studies comparing the effects of inhaled crystalline silica and titanium dioxide have shown a correlation between the release of the macrophage derived cytokine IL-1 and granuloma formation (Driscoll and Maurer, 1991). Consequently these workers suggested that IL-1 might be a useful biomarker for granuloma formation.

Localised, angiocentric granulomas of foreign body type, clustered around pulmonary arteries and arterioles and occasionally alveolar capillaries and venules

Fig. 30. Foreign body granuloma in the lung of a Sprague-Dawley rat given repeated intravenous doses of a soluble synthetic polymer. (HE, x100.)
also develop following intravenous injection of relatively insoluble polysaccharides or other polymers (Jonson et al., 1984). Characteristic epithelioid and large, foreign body type giant cells efface the smaller vessels although overt necrosis is not usually observed (Fig. 30).

**Pigment**

Haemosiderin-laden macrophages accumulate in the alveoli of laboratory animals in association with chronic pulmonary congestion and haemorrhage. Similar changes occur in man particularly in congestive cardiac failure where the haemosiderin-laden macrophages are frequently termed ‘heart failure’ cells.

The lungs of non-human primates are especially liable to contain alveolar, perivascular and peribronchial aggregates of macrophages laden with various brown pigments. Iron-containing pigments have been associated with the inflammatory changes produced by simian lung mites (*Pneumonyssus simicola*) which are prevalent in many non-human primates. In addition, lungs from some primate colonies may show perivascular and peribronchial collections of brown-grey macrophages containing highly refractive spicules and plates composed of high concentrations of silica (Dayan et al., 1978; Kim and Cole, 1987). It has been shown that in Old World primates including rhesus and cynomologous monkeys, this pigment contains fossil diatomaceous material, compatible with the concept that the animals inhale dusts containing diatoms and other silicon fragments to which they are exposed in their semi-arid, natural habitats (Dayan et al., 1978).

**Fibrosis**

Chronic lung injury from a variety of different causes is frequently associated with the development of pulmonary fibrosis characterised by the replacement of the normal pulmonary structure by a thickened collagenous matrix with consequent reduction in the capacity for gas exchange. Regardless of the inciting agent or agents, the fibrogenic process appears to be generally characterised by disruption of normal the alveolar-capillary structure, leakage of exudate from the vascular compartment into the airspaces, with subsequent invasion by inflammatory cells and fibroblasts and excess matrix formation. Studies in laboratory animals with different fibrogenic agents as well as in humans have suggested that central to pulmonary fibrogenesis is increased production of tumour necrosis factor-α (TNF-α) by macrophages (Phan and Kunkel, 1992; Driscoll et al., 1994; Piguet and Vesin, 1994; Warheit et al., 1995; Uhl et al., 1998). This cytokine is not only a mitogen for fibroblasts but also a potent activator and chemoattractant for macrophages, capable of stimulating release of other cytokines and inducing expression of adhesion molecule expression on endothelial cells (Derynck, 1992; Warheit et al., 1995; Uhl et al., 1998). Moreover, it has been shown that TNF-α receptor knockout mice appear protected from the fibroproliferative effects of inhaled asbestos (Liu et al., 1998).
Pulmonary fibrosis is a common sequel of chronic lower respiratory tract inflammation. It may be associated with, or preceded by interstitial pneumonitis, characterized by infiltration by lymphocytes, plasma cells and macrophages with scattered polymorphonuclear cells (Singer et al., 1986). Focal pulmonary fibrosis occurs spontaneously in laboratory animals, although this is usually most prevalent in dogs and non-human primates as a response to chronic infestation by parasites which are not easily eliminated during breeding.

In man, conditions leading to pulmonary fibrosis vary widely. They include infections, shock lung syndrome, ionising radiation, inhalation of irritant particulate matter, exposure to antigens or excessive amounts of oxygen as well as the results of the toxicity of paraquat and a range of both cytotoxic and non-cytotoxic therapeutic agents which cause pulmonary parenchymal injury (Johnson et al., 1979; Hollinger, 1993).

The principle therapeutic agents which produce pulmonary fibrosis in both man and laboratory animals are anticancer drugs. Bleomycin, a glycopeptide preparation derived from *Streptomyces verticillus* is the best known example but pulmonary fibrosis is also associated with the clinical use of a number of other anticancer agents including 1,3-bis-(2-chloroethyl)-1-nitrosourea (BCNU or carmustine), cyclophosphamide, busulphan, mitomycin C and methotrexate (Gutin et al., 1976; Weiss and Muggia, 1980; Kehrer and Kacew, 1985; Hollinger, 1993; Malik et al., 1996). A wide range of non-cytotoxic drugs is also reported to produce pulmonary fibrosis in humans (Hollinger, 1993; Tomioka and King, 1997).

The precise mechanisms involved in the induction of pulmonary fibrosis by antineoplastic drugs in man are poorly understood. The true incidence for a particular drug is also difficult to estimate because of confounding factors in cancer patients, such as concomitant administration of several drugs, radiation and oxygen therapy, diffuse pulmonary cancer and opportunities infections. It is probable that drug-induced fibrosis is accentuated by concomitant administration of several antineoplastic agents, radiation therapy, hyperoxia, pre-existing pulmonary damage and age of the patient. Severity is often related total dose of drug received (Kehrer and Kacew, 1985).

Bleomycin is associated with the development of interstitial pneumonia and pulmonary fibrosis in clinical use (Luna et al., 1972) and this can also be induced in experimental animals. The histopathological appearances of bleomycin-induced pulmonary fibrosis in patients are in many instances different from those seen in laboratory animals because the lungs of patients treated with beomycin are modified by factors such as primary neoplastic disease, multiple drug and radiation therapy as well as secondary pulmonary infections, interstitial pneumonitis and fibrosis. It has been postulated that TNF-α is an important mediator in the development of bleomycin-induced fibrosis (Piquet and Vesin, 1994).

Beagle dogs given cycles of bleomycin by the intravenous route for periods of up to 26 weeks developed anorexia, weight loss, a variety of epithelial lesions as well as focal interstitial pneumonia and fibrosis (Thompson et al., 1972). In the dog study reported by Thompson et al. (1972), the lesions were those of focal
interstitial pneumonia and fibrosis characterised by increased elastic fibres, reticulin, collagen and acid mucosubstances. The lesions were situated predominantly in the pleural and subpleural zones, suggestive of a potentiating effect of friction between the pleural surfaces. Histologically the lesions resembled those produced by larvae migrans in the dog. Similar histological changes have also been described in both rats and mice treated with bleomycin by both the intravenous and intratracheal route (Thrall et al., 1979; Lindenschmidt et al., 1986). As fibrosis is such a consistent change, bleomycin-treated rodents have been extensively employed as a model for pulmonary fibrosis. Early changes include mild, diffuse increase in interstitial lymphocytes, macrophages, a few polymorphonuclear cells and perivascular or interstitial oedema. After about a week, interstitial infiltrates also comprise fibroblasts with early collagen deposition, associated with proliferation of macrophages and type II pneumocytes (Thrall et al., 1979; Lindenschmidt et al., 1986). Subsequently, the amount of interstitial collagen increases, with eventual scarring and collapse of lung tissue in proportion to the cumulative dose given (Brown et al., 1988). Immunohistochemical and ultrastructural study of rats and mice treated with bleomycin shows large accumulation of immune-reactive laminin and reduplication of the basement lamina within the thickened alveolar walls (Singer et al., 1986).

In bleomycin-treated rats changes in pulmonary capillary structure have been demonstrated by three-dimensional scanning electron microscopy. Treatment produces irregular alveolar and pleural capillaries with increased diameter and decreased branching (Schraufnagel et al., 1986).

Certain strains of mice have been shown to possess greater sensitivity to bleomycin fibrogenesis. The C57BL/6 strain produces a greater fibroblastic response than DBA/2 and Swiss mice and the BALB/C strain demonstrates a particularly poor fibroblastic response (Schrier et al., 1983).

Therapeutic use of cyclophosphamide is also occasionally associated with the development of pulmonary interstitial fibrosis (Weiss and Muggia, 1980; Malik et al., 1996). It appears to be associated with two forms of pathology: an early-onset pneumonitis and a late onset progressive pulmonary fibrosis (Malik et al., 1996). Similar changes have been less easy to reproduce in laboratory animals. When mice were sequentially examined for periods of up to 1 year after a single intravenous dose of 100 mg/kg of cyclophosphamide, only slight pulmonary interstitial thickening and hypercellularity was observed in association with progressive multifocal accumulation of intra-alveolar macrophages (Morse et al., 1985). However, these changes were also accompanied by a progressive increase in pulmonary hydroxyproline content and a decrease in pulmonary compliance with time in treated animals compared with controls. The changes were amplified by exposure to 70% ambient oxygen.

The bronchiolitis, alveolar septal inflammation and fibrosis induced by gold therapy in patients with rheumatoid arthritis is of interest as it is probably immune-mediated. This condition is associated with peripheral eosinophilia and drug-induced alterations to the immune system (Tamoika and King, 1997).
Emphysema

Emphysema is characterised by abnormal, permanent enlargement of airspaces distal to terminal bronchioles, accompanied by destruction of their walls without obvious fibrosis.

Three principle types, centriacinar, panacinar and distal acinar emphysema are recognised in man. Enlargement of air spaces as a result of congenital factors or fibrous scarring are grouped separately and not regarded as true emphysemas (Snider et al., 1986).

Although emphysema is occasionally observed as an age-related spontaneous change in laboratory rodents (Levame, 1980), several experimental rodent emphysema models have been developed, using intratracheal instillation of proteolytic enzymes papain, pancreatic and neutrophil elastase. This gives rise to histological appearances resembling panacinar emphysema in man. Elastase enters the alveolar wall via type I pneumocytes and spreads through the lung interstitium producing degradation of elastin fibres with associated oedema and cellular exudate. Elastase then enters the blood where it is inactivated and cleared by the pulmonary circulation. Eventually the alveolar exudate clears and air space enlargement occurs (See review Snider et al., 1986). Intravenous administration of elastase may also induce emphysema but higher doses are required.

Irritant gases notably oxides of nitrogen are also capable of inducing changes in the lungs of laboratory rats and hamsters following long term exposure which resemble mild human, centrilobular emphysema (Juhos et al., 1980; Lam et al., 1985).

Phospholipidosis (lipidosis)

A variety of different names have been applied to membrane-bound, acid phosphatase-positive cytoplasmic inclusions with a lamella or crystalloid ultrastructural matrix. These include myeloid bodies, myelinoid bodies, myelin figures or myelinosomes. These lysosomal inclusions are seen in small numbers in a variety of normal cell types, but they accumulate in various organs following administration of a wide variety of xenobiotics.

A characteristic and generalised accumulation of these cytoplasmic inclusions is reported in laboratory animals following repeated administration of numerous amphiphilic, cationic drugs. Generalised accumulation of lysosomal cytoplasmic bodies is generally called phospholipidosis, a term coined to describe the tissue accumulation of phospholipids (Shikata et al., 1972). A number of workers have reviewed the drugs that cause phospholipidosis in laboratory animals (Lüllmann et al., 1975; Reasor, 1981; Hollinger, 1993; Halliwell, 1997). Inducing agents are of quite different therapeutic classes but they usually share structural features including a hydrophilic cationic side chain, a primary, secondary or tertiary amine and a hydrophobic region that is usually an aromatic ring or ring system. As this structural pattern renders these molecules amphphilic,
these drugs probably bind with polar lipids by means of electrostatic and hydrophobic forces (Lüllmann et al., 1975). This leads to formation of drug-lipid complexes which are poorly degraded by lysosomal enzymes and which accumulate in the cell cytoplasm to form the inclusions described above. As the binding is not covalent, its reversibility depends on the dissociation rate constant under the particular intracellular conditions and drug concentration achieved.

Predictions of this activity based on molecular structure have shown reasonably good correlation with the ability of compounds to produce phospholipidosis in cultured rat peritoneal macrophages (Lüllmann-Rauch, 1979). This correlation has been less good when whole animal data are evaluated, presumably because of differences in drug disposition in blood and tissues. Actual examples include the anorectic drug, chlorphentermine, tricyclic antidepressants, inhibitors of cholesterol biosynthesis such as triparanol, the antihistamine, chlorcyclizine and its analogues and the antioestrogen, tamoxifen, chloroquine, the cardiovascular drugs amiodarone, 4, 4'-diethylamino-ethoxyhexestrol and perhexiline (Lüllmann et al., 1975; Lüllmann and Lüllmann-Rauch, 1981; Lüllmann-Rauch, 1979). Many tissues and organs may develop the cytoplasmic inclusions including lymphoid cells, liver, pancreas, endocrine tissue, nervous system, muscle cells, eyes and particularly lungs.

Fig. 31. Lung from a Wistar rat given tamoxifen for over a year. The alveoli contain large macrophages with pale granular cytoplasm typical of phospholipidosis. There is no evidence of inflammation or parenchymal damage. (HE, x80.)
The lungs appear especially vulnerable to drug-induced phospholipidosis, possibly because macrophages are in very close proximity to blood-borne agents (Reasor, 1981). The continuous uptake of phospholipid-rich surfactant material from the alveoli by macrophages leads to excessive accumulation of phospholipids when their catabolism is impaired (Lüllmann et al., 1975). The fact that lungs are commonly affected is a potentially useful diagnostic feature because in many organs phospholipidosis can be extremely difficult to recognise by light microscopy of haematoxylin and eosin-stained sections. Although the changes in the lungs are not specific for drug-induced phospholipidosis, an increase in the number of lipid-contain lung macrophages in treated animals compared with controls is relatively easy to detect and provides a simple way for the pathologist to screen for this effect.

In severe generalised phospholipidosis in rats, the lungs show irregular pale grey or yellowish patches of discoloration of the pleura and parenchyma. This is a result of patchy or confluent aggregates of large, pale, foamy macrophages, free lying or packed in alveoli and commonly accompanied by granular, extracellular material. Their abundant cytoplasm shows a vacuolated appearance in which fine eosinophilic granules are sometimes visible. The nuclei are rounded and centrally located structures of variable size. Multi-laminated cells are also occasionally seen, as are vacuolated cells firmly attached to alveolar walls, probably pneumocytes. These foamy cells stain typically for phospholipids (e.g. acid haematin), although neutral lipid may also be present and stain with oil red O.

Semi-thin plastic embedded sections stained with toluidine blue allow better characterisation of phospholipidosis in all organs including the lungs. The macrophages in the air spaces contain unmistakable dense, dark round cytoplasmic inclusions of variable size, some over 5 mm diameter (Heath et al., 1985). Plastic embedded sections also show the inclusions in other pulmonary cells including pneumocytes attached to the alveolar walls from which they can be seen discharging into the alveolar spaces.

As in other organs affected by phospholipidosis, ultrastructural examination reveals dense, multi-lamellar membranes and numerous heterogeneous dense bodies of lysosomal origin. These bodies need to be distinguished from membranous bodies that form as a result of fixation for ultrastructural study. Lipids tend to leach out and become hydrated to form myelinoid membranes during glutaraldehyde fixation. These structures are subsequently fixed by osmium to give rise to electron-dense membranous figures both outside and inside cells particularly in mitochondria where they may be mistaken for pathological lesions (Ghadially, 1980; Lüllmann-Rauch, 1979; Costa-Jussà et al., 1984; Robinson et al., 1985).

The lamella patterns seen in phospholipidosis may be simple alternating dense and clear lines spaced at 4–5 nm, or more complex arrangements of clear and dense lines (Lüllmann-Rauch, 1979). The other typical crystalloid inclusions of hexagonal aggregates of tubular subunits seen in other organs are not usually found in the lungs. The significance of these various forms is uncertain but they probably represent the various phases in which phospholipids exist and are in-
fluenced by proportions of lipids present (Lüllmann-Rauch, 1979). Electron microscopic examination reveals that not only are pulmonary macrophages affected by these changes but that inclusions may be present in pneumocytes types I and II, pulmonary capillary endothelial cells, smooth muscle cells, bronchiolar epithelium and occasionally neutrophils (Lüllmann-Rauch and Reil, 1974; Costa-Jussà et al., 1984; Robinson et al., 1985). The changes are typically still visible several weeks after withdrawal from treatment with the offending agent.

Although the extent of pulmonary phospholipidosis in the lungs may vary between dosage regimen and species, studies with chlorphentermine, 4, 4’-diethylaminooethoxyhexestrol and amiodarone have indicated that similar cytological and ultrastructural changes occur in most laboratory animal species studied including rats, mice, hamsters, guinea pigs, rabbits and dog (Lüllmann-Rauch and Reil, 1974; de la Iglesia et al., 1975; Costa-Jussà et al., 1984; Mazué et al., 1984).

What are the implications for humans of drug that induce phospholipidosis in laboratory animals? Although not all agents which produce phospholipidosis in animals have been studied in man, only very few drugs, which produce phospholipidosis in animals, are capable of inducing phospholipidosis in human clinical practice (Hollinger, 1993). Agents such as chloroquine, 4,4’-diethylamindethoxyhexestrol and amiodarone which have been shown to produce phospholipidosis in man also induce frank cellular damage in the same organs. It remains unclear whether the phenomena of phospholidosis is causally related to cellular damage in humans, so that the finding of phospholipidosis in animal studies with a novel drug requires careful assessment on a case by case basis with respect to its implications for the safety of humans.

An example of this issue is the iodinated benzofuran derivative amiodarone, a potent antiarrhythmic drug effective against ventricular arrhythmia. Lung toxicity continues to be a problem in patients treated for cardiac arrhythmias with this drug. Not only does phospholipidosis occurs in a wide variety of organs in laboratory animals treated with amiodarone (Mazué et al., 1984; Riva et al., 1987) but also in liver, peripheral nerve cells, skin, lymphoid cells and lungs in man at therapeutic doses (Costa-Jussà et al., 1984; Shepherd et al., 1987; Fan et al., 1987). Whereas phospholipidosis induced by amiodarone in the lungs of rodents is not associated with fibrosis or significant functional alterations (Riva et al., 1987; Heath et al., 1985), pulmonary interstitial fibrosis occurs in association with phospholipidosis in man (Costa-Jussà et al., 1984).

Several theories have been proposed for the pulmonary alveolitis and interstitial fibrosis in humans. The weight of evidence to date suggests that the accumulation of lipid-laden histiocytes is not causally related to the alveolitis or pulmonary fibrosis (Reasor and Kacew, 1996). Cytotoxicity, possibly through the metabolite desethylamiodarone has been proposed and an immune-mediated mechanism has been postulated, possibly favoured by the binding of drug to components of pulmonary tissue (Fan et al., 1987). It is also possible that pulmonary disease results in particular patients from an interaction of several mechanisms and metabolic factors (Reasor and Kacew, 1996).
Despite undoubted differences in tissue and species sensitivity to development of phospholipidosis, dose, drug disposition, metabolism and elimination and the degree of tissue exposure to drug are important considerations in safety assessment of drugs that produce phospholipidosis in laboratory animals. Although phospholipidosis is more likely to occur at high doses employed in toxicity studies than at lower therapeutic doses used in man, it has been suggested that this may be offset by faster elimination of the drug, characteristic of small laboratory animals (Lüllmann et al., 1975). The potential for drugs to accumulate in critical tissues such as eye and heart are especially important when drugs are administered for long periods of time particularly as tissue/plasma ratios of some amphiphilic drug may exceed 100, following repeated administration (Lüllmann et al., 1978). Consequently, the implications for humans of drugs that induce phospholipidosis in laboratory animals can only be assessed on a case by case basis, with due consideration of mechanism, drug disposition and clinical risk-benefit analysis.

It is important to underline that similar morphological changes can also result from treatment with compounds that are not cationic amphiphilic structures. An example of this is the induction of lysosomal inclusions in the lungs of rat and dogs by the antibiotic, erythromycin (Gray et al., 1978). Hook (1991) has reviewed the range of agents that can increase phospholipid levels in the lung, which include oxidant gases and insoluble particles such as silica.

The accumulation of foamy macrophages in the alveolar spaces also occurs as a spontaneous change in ageing rats (Yang et al., 1966). In contrast to drug-induced changes, the spontaneous lipidosis occurs sporadically in older rats and is observed in both controls and treated animals. Drug-induced phospholipidosis occurs within a period of several months, during which lungs of control animals remain fairly free of spontaneous, foam-cell accumulation.

**Hyperplasia**

Various forms of hyperplasia are found in the airways and lungs of laboratory animals. The mucosal surface of the bronchi may show hyperplasia of the goblet cells and squamous hyperplasia or metaplasia. The cells lining the terminal bronchiole and alveolus may also show hyperplasia and squamous metaplasia. These changes have been summarised for the rat in the classification of the Society of Toxicologic Pathologists (Schwartz et al., 1994) and the International Agency for Research on Cancer (Mohr, 1992).

*Goblet cell hyperplasia, goblet cell metaplasia (mucous cell hyperplasia)*

Goblet cell hyperplasia is a well recognised response of the mucosa of conducting airways to chronic inflammation and inhalation of irritant substances such as cigarette smoke and sulphur dioxide (Reid, 1963; Jones et al., 1973; Coggins et al., 1980). It has also been reported to occur in the hamster emphysema model.
in which human neutrophil elastase is instilled by the intratracheal route (Christensen et al., 1987).

The degree of goblet cell hyperplasia is dictated by the severity and duration of the irritation or inflammatory process. However, species differences exist because the airways of laboratory animals are variably endowed with goblet cells and submucosal mucous glands. For instance, the normal rat has more goblet cells lining the airways than either mouse or hamster (Reid, 1963).

Florid cases of goblet cell hyperplasia are characterised histologically by thickening and pseudostratification of the tracheal or bronchial mucosa by a population of tall, mucus secreting cells with abundant pale cytoplasm. In addition, goblet cells extend further down the airways than in normal animals and mucus may fill or distend the airways or impact in the alveoli (Reid, 1963). In less florid cases, a simple increase in the number of goblet cells may be found without other structural change (Coggins et al., 1980). The factors controlling these alterations are uncertain but is has been suggested that increased mitotic activity as well as cell conversion, probably by metaplasia of serous or Clara cells to mucous cells is involved (Bolduc et al., 1981; Christensen et al., 1987).

This type of goblet cell hyperplasia of the lining epithelium may be accompanied by an increase in size of the underlying submucosal glands. This has clearly been demonstrated in patients with chronic bronchitis and in rats where submucosal glands are normally quite prominent (Reid, 1960,1963).

Spontaneous goblet cell metaplasia of the pulmonary alveolar epithelium has been described in the rat as a localised mass comprising distended alveoli and bronchioles filled with mucin and lined by non-ciliated epithelial cells with numerous goblet cells, presumably of developmental origin (Nagai, 1994).

Pharmacological agents can induce goblet or mucous cell hyperplasia. Rats given 6 or 12 daily injections of isoprenaline, a non-selective β-receptor agonist, showed a dose- and time-dependent increase in the number and size of alcian blue-positive goblet (mucous) cells as well as serous cells in the tracheal and bronchial mucosa. This was associated with an increase in length, width and depth of submucosal glands (Sturgess and Reid, 1973). Similar changes were produced by pilocarpine, although both alcian blue and PAS positive cells were increased in number following this agent, suggesting that pilocarpine induced both acid and neutral glycoprotein secretion.

Critical comparison of the distribution of these changes in the rat following isoprenaline, with those of salbutamol, pilocarpine and tobacco smoke showed that there were regional differences in the distribution of these changes in the airways (Reid and Jones, 1983). Isoprenaline produced a greater increase in secretory cells in peripheral airways than tobacco smoke which itself produces a greater increase in mitotic activity. Isoprenaline and pilocarpine produced a more diffuse change than the more selective β-agonist, salbutamol.

The changes induced by these therapeutic agents are presumably the result of their pharmacological activity (Reid and Jones, 1983). Sturgess and Reid, 1973 showed that the changes in the rat were accompanied by hypertrophy of the pancreas, submaxillary and parotid salivary glands. See Digestive System, Chapter VII.
Unlike the rat and mouse, the hamster appears predisposed to develop minor multifocal epithelial hyperplasia of the tracheal and bronchial mucosa spontaneously with advancing age. These changes are flat or polypoid in nature and are composed of clear cells and goblet cells (Pour et al., 1976, 1979).

**Squamous hyperplasia, squamous metaplasia**

The epithelium of the bronchi shows squamous metaplasia in response to chronic irritation or injury characterised by three or more layers of epithelial cells with abundant eosinophilic cytoplasm with prominent cell boundaries. It may be associated with degenerative alterations to the mucosa and goblet cell hyperplasia. Squamous metaplasia can also develop in the alveolar parenchyma also related to prolonged damage such as produced by large burden of inhaled irritant or insoluble dusts. The metaplasia is also characterised by the presence of several layers of flattened epithelial cells showing squamous differentiation. The classification of these cystic keratinising lesions in the rat lung in association with large burdens of particulate matter has been the subject of a specialist workshop (Boorman et al., 1996). The term pulmonary keratinising cyst was recommended for large cystic lesions lined by non-neoplastic squamous epithelium without excessive proliferative change.

**Hyperplasia, bronchiolo-alveolar (type II cell hyperplasia)**

Hyperplasia may involve the lining epithelium of the alveoli or bronchioli. This form of hyperplasia has been termed alveolar hyperplasia, adenomatosis, alveolar bronchiolisation or epithelialisation. In rats, this form of hyperplasia occurs spontaneously. It can be induced by infections and administration of xenobiotics in rats (Coleman et al., 1977; Goodman et al., 1979; Kroes et al., 1981; Greaves and Facchin, 1992), mice (Ward et al., 1974, 1979) and hamsters (Rehm et al., 1989).

Whether alveolar hyperplasia results from hyperplasia of type II cells or bronchiolar cells migrating into alveoli is disputed (Rehm et al., 1989). Histologically, the lesions consist of localised but unencapsulated foci of hyperchromatic regular, cuboidal or columnar cells investing airspaces without appreciable distortion of alveolar walls.

**Neuroendocrine hyperplasia**

Another form of hyperplasia, which is well described in hamsters, is neuroendocrine hyperplasia. Although small aggregates of neuroendocrine cells (neuroepithelial bodies) are found at various levels of the bronchi and bronchioli in the normal hamster, administration of nitrosamines and 4-nitroquinoline 1-oxide can produce neuroendocrine hyperplasia (Reznik-Schüller, 1977; Linnoila et al., 1981; Ito et al., 1986). Hyperplastic lesions are recognisable as groups of non-ciliated cuboidal, oval or columnar cells located in the bronchial or bronchiolar epithelium. They contain argyrophilic granules that show immunoreactivity for
corticotrophin (ACTH) and neurone-specific enolase (Ito et al., 1986; Linnoila et al., 1981). Ultrastructural examination reveals the presence of dense-core cytoplasmic granules of APUD type (Reznik-Schüller, 1977; Linnoila et al., 1981; Ito et al., 1986).

**Neoplasia**

The common lung neoplasm in man, bronchogenic squamous carcinoma, is only occasionally observed as a spontaneous pulmonary neoplasm in laboratory rodents including rats (Goodman et al., 1979). There is no good experimental model for small cell (oat cell) cancer which comprises about 25% of all lung cancers in man and which believed to be of neuroendocrine derivation (Becker and Gazder, 1985).

By far the most common primary pulmonary neoplasms found in laboratory rats, mice and hamsters are adenomas and adenocarcinomas. These appear to develop from the bronchiolar or alveolar epithelium although their precise histogenesis is somewhat disputed. Although spontaneous squamous neoplasms are uncommon in rodents there has been the subject of debate because cystic keratinising lesions can be induced in rats by high burdens of particulate material in the lungs (Boorman et al., 1996). Pleural mesotheliomas and mesenchymal neoplasms also occur in these species but are rare. Mesenchymal tumours have similar histological features to those in soft tissues and mesotheliomas may show either epithelial or mesenchymal differentiation or both.

**Rat**

In most rat strains alveolar or bronchiolar neoplasms occur spontaneously in relatively small numbers, but morphologically identical neoplasia can be induced by administration of chemical carcinogens (Reznik-Schüller and Reznik, 1982). The most common are classified as bronchiolo-alveolar adenoma (pulmonary adenoma) and bronchiolo-alveolar carcinoma. The National Toxicology Program database on control Fischer 344 rats used in carcinogenicity studies indicates an overall percentage of less than 3% of animals with bronchiolo-alveolar adenomas and less than 1% with bronchiolo-alveolar carcinomas (Haseman et al., 1998). However the range of bronchiolo-alveolar adenomas was between 0 and 14% in this series.

Histologically, bronchiolo-alveolar tumours are mostly small, discrete, rounded nodules located in the lung parenchyma and composed of fairly uniform cells with moderately hyperchromatic nuclei arranged in solid (alveolar), tubular, papillary or mixed growth patterns. They usually compress surrounding tissues without infiltration or metastatic spread (adenoma) although loss of differentiation, infiltration and spread to adjacent tissues can occur (adenocarcinoma).

Ultrastructural study of bronchiolar-alveolar neoplasia in Fischer 344 rats has shown the presence of osmiophilic, lamellated inclusion bodies similar to those found in alveolar type II cells. Therefore it has been suggested that the neoplasms are derived from this cell type (Reznik-Schüller and Reznik, 1982).
However, lamellar inclusions can occur in cells of other types, including Clara cells (Rehm et al., 1989).

Pulmonary squamous carcinoma occurs but is a very uncommon spontaneous neoplasm in the rat (Schwartz et al., 1994). The large proliferative but benign cystic lesions found in the lungs of rats following accumulation of large amount of particulate matter have been termed pulmonary cystic keratinising epitheliomas for they have been regarded as benign neoplasms. When these lesions show evidence of tissue invasion they are regarded as pulmonary squamous cell carcinomas (Boorman et al., 1996). Similar lesions are very occasionally reported as spontaneous lesions (Rittinghausen and Kaspareit, 1998).

**Mice**

Analogous neoplasms are found more commonly in most strains of laboratory mice used in carcinogenicity bioassays although there is considerable reported variation in incidence. They are common in strain A mice where they are observed in low frequency at 3–4 months of age and incidences reach nearly 100% by 24 months of age (Stoner and Shimkin, 1982). Fewer, but significant numbers are found in B6C3F1 mice, although considerable inter-laboratory variation in the presence of these neoplasms is reported (Tarone et al., 1981). The National
Toxicology Program data base on control B6C3F₁ mice used in carcinogenicity studies indicates an overall percentage of about 16% of males and 6% of females with bronchiolo-alveolar adenomas but only about 5% and 2.5% respectively with bronchiolo-alveolar carcinomas (Haseman et al., 1998). However the range of bronchiolo-alveolar tumour varied considerable between studies in this series. Even in the same laboratory, mice housed under similar conditions show variation in incidence in these neoplasms with time. The incidence of lung adenomas and adenocarcinomas occurring in CD-1 mice used as controls in 18-month carcinogenicity bioassays in the same laboratory under similar conditions for a period of 3 years varied from between 19 to 36% in males and from 6 to 16% in females (Faccini et al., 1981). By contrast, some strains of mice such as the C5781/10J strain show a very low predisposition to lung adenomas (Tucker, 1985). By contrast to bronchiolo-alveolar neoplasms, very few squamous carcinomas are reported in most series of mouse studies.

Histologically, pulmonary tumours of this type in mice are generally small, sharply circumscribed nodules composed of fairly uniform, closely packed columns of cuboidal or columnar cells arranged in tubular or papillary structures with scanty fibrovascular stroma (Figs. 32 and 33). They may be less well differentiated with cellular pleomorphism and show intrabronchial growth, invade lung parenchyma and produce metastatic spread (Stewart et al., 1979).
The precise histogenesis of mouse pulmonary adenomas and adenocarcinomas is disputed. On the basis of sequential light and electron microscopic study of pulmonary adenomas induced in Bagg-Webster Swiss mice by transplacental exposure to ethylnitrosourea, it has been suggested that they develop from either alveolar type II cells or Clara cells (Kauffman et al., 1979; Kauffman, 1981).

Careful, stepwise analysis using light microscopic and electron microscopic examination showed that adenomas fell into three principle groups. Some were composed of solid growths of uniform cuboidal cells with expanding margins limited to alveolar septae (alveolar pattern). These cells contained concentrically arranged cytoplasmic lamellar bodies and abundant, large mitochondria similar to mitochondria found in alveolar type II cells. Other patterns were tubular or papillary in type, each being composed of cuboidal cells showing histological and ultrastructural features of Clara cell differentiation. Based on the time sequence of development, it was suggested that tumours with a tubular arrangement evolved into papillary adenomas in this experimental model (Kauffman, 1981).

Immunocytochemical studies of chemically-induced and spontaneous pulmonary neoplasia in B6C3F1, BALB/c or A strain mice have shown that the majority of adenocarcinomas, including those showing papillary patterns, contained immune-reactive surfactant apoprotein, typical of type II antigens suggesting that most neoplasms are of alveolar type II derivation (Ward et al., 1985). Gunning et al. (1991) examined a range of adenomas in strain A mice and showed that the proportion of tumours with papillary and solid/alveolar growth patterns varied with the inducing agent. This suggests certain biological differences exist between the two histological subtypes. Perhaps, these findings are not surprising in view of the pluripotential nature of lung precursor cells and the fact that differentiation may proceed along different pathways during tumour progression, which have the potential to be modified by xenobiotics.

The usual high incidence and the inherent variability of pulmonary adenomas and adenocarcinomas in conventional mouse carcinogenicity bioassays sometimes gives rise to statistically significance differences between control and treatment groups. As frank genotoxic carcinogens also increase the prevalence of similar neoplasms in strain A mice, there is considerable risk in over-interpretation of such group differences in conventional mouse bioassays. In the analysis of group differences, it is important that consideration is given to tissue sampling procedure, age-standardisation, historical control incidence, effects on food intake, and the results of mutagenicity studies and carcinogenicity bioassays in other rodent species.

For instance, in a carcinogenicity bioassay in which CF1 mice were treated for 80 weeks with the synthetic analgesic tilidine fumarate, a statistically significant difference (p < 0.01) was reported in the incidence of lung adenocarcinomas between the top dose female group (24%) and concurrent controls (10%) (McGuire et al., 1986). It was argued that group differences did not indicate tumorigenic potential of tilidine fumarate on the basis that the incidence in the high dose group was within the historical control range (27%) and that there
was no tumorigenic effect evidence in an analogous 104-week rat carcinogenicity study.

A more difficult evaluation concerned metronidazole, a nitroimidazole, which is an important therapeutic agent active against anaerobic organisms and trichomonas species. Administration of this compound led to an increased incidence of pulmonary adenomas and carcinomas in three separate mouse carcinogenicity bioassays (Rustia and Shubik, 1972; Roe, 1983).

The analysis of these findings was complicated by evidence that metronidazole shows mutagenic activity in bacterial assays using some strains of *Salmonella typhimurium*. Roe (1983) argued that the risk to man was slight because the increase in prevalence in pulmonary tumours was likely to be a result of changes in nutritional status of the mice through the effect of metronidazole on gut flora, as similar differences could occur between ad libitum fed mice and those fed the same but restricted diet. He also suggested that the positive findings in bacterial mutagenesis assays were an inherent part of the antibacterial activity of metronidazole as a result of nitroreduction that does not occur in normal mammalian tissues. This conclusion was supported by negative effects in hamster carcinogenicity bioassays and supplemental genotoxicity tests as well as lack of excess cancer risk in women followed up for 10 years or more (Roe, 1983).

**Strain A mouse pulmonary tumour bioassay**

The common occurrence of lung adenomas in strain A mice has been utilised in the development of a quantitative bioassay for carcinogenic activity. This followed the demonstration that administration of carcinogens such as 3-methylcholanthrene to this strain could significantly increase the incidence of pulmonary adenomas within periods of up to 6 months (Shimkin, 1940). Over many years the strain A mouse pulmonary tumour assay has been used to test a large number of chemicals of different classes including polycyclic hydrocarbons, nitrosamines, food additives, alkyl halides, metals and chemotherapeutic agents (Stoner et al., 1973; Gunning et al., 1991). However, as with many biological systems, interlaboratory agreement in the strain A test system and correlation with 2-year carcinogenicity study data and genotoxicity results have been shown to be poor, so particular care is needed in the interpretation of this test (Maronpot et al., 1986). Nevertheless, it may serve to help in the ranking of tumorigenic activity of some anticancer drugs provided cognisance is given to drug stability, route of administration and vehicle as well as species differences in metabolism and drug disposition.

**Hamsters**

Hamsters develop lung adenomas spontaneously in small numbers with advancing age. They are composed of uniform cylindrical cells similar to those found in bronchial epithelium or goblet cells showing distinct mucus production (Pour et
al., 1976, 1979; Mohr and Ketkar, 1980). An immunohistochemical study of similar pulmonary neoplasms induced in hamsters by N-nitrosodiethylamine has shown the presence of Clara cell antigen in early phase of development but as the tumours developed they became more squamous in type and showed immunoreactivity for cytokeratins (Rehm et al., 1989). A predominantly Clara cell origin was suggested for these neoplasms.

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