

The Nose Revisited: A Brief Review of the Comparative Structure, Function, and Toxicologic Pathology of the Nasal Epithelium

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ABSTRACT

The nose is a very complex organ with multiple functions that include not only olfaction, but also the conditioning (e.g., humidifying, warming, and filtering) of inhaled air. The nose is also a “scrubbing tower” that removes inhaled chemicals that may be harmful to the more sensitive tissues in the lower tracheobronchial airways and pulmonary parenchyma. Because the nasal airway may also be a prime target for many inhaled toxicants, it is important to understand the comparative aspects of nasal structure and function among laboratory animals commonly used in inhalation toxicology studies, and how nasal tissues and cells in these mammalian species may respond to inhaled toxicants. The surface epithelium lining the nasal passages is often the first tissue in the nose to be directly injured by inhaled toxicants. Five morphologically and functionally distinct epithelia line the mammalian nasal passages—olfactory, respiratory, squamous, transitional, and lymphoepithelial—and each nasal epithelium may be injured by an inhaled toxicant. Toxicant-induced epithelial lesions in the nasal passages of laboratory animals (and humans) are often site-specific and dependent on the intranasal regional dose of the inhaled chemical and the sensitivity of the nasal epithelial tissue to the specific chemical. In this brief review, we present examples of nonneoplastic epithelial lesions (e.g., cell death, hyperplasia, metaplasia) caused by single or repeated exposure to various inhaled chemical toxicants. In addition, we provide examples of how nasal maps may be used to record the character, magnitude and distribution of toxicant-induced epithelial injury in the nasal airways of laboratory animals. Intranasal mapping of nasal histopathology (or molecular and biochemical alterations to the nasal mucosa) may be used along with innovative dosimetric models to determine dose/response relationships and to understand if site-specific lesions are driven primarily by airflow, by tissue sensitivity, or by another mechanism of toxicity. The present review provides a brief overview of comparative nasal structure, function and toxicologic pathology of the mammalian nasal epithelium and a brief discussion on how data from animal toxicology studies have been used to estimate the risk of inhaled chemicals to human health.

Keywords. Nose; comparative anatomy; nasal toxicology and pathobiology; inhaled toxicants; airway epithelium.

INTRODUCTION

In recent years there has been an explosion of new scientific information on the structure and function of the nose at the macroscopic, microscopic and molecular levels of analysis. This often unappreciated part of the mammalian upper airway has recently been the focus of reported research efforts in a variety of diverse scientific disciplines such as pharmacology, neuroscience, mathematical modeling, toxicology, and even paleontology. The tremendous advances made over the past decade in our knowledge of how animals smell (olfaction) was recently highlighted world-wide when Drs. Linda Buck and Richard Axel were awarded the 2004 Nobel Prize in physiology or medicine for their pioneering work on the cellular and molecular mechanisms of olfaction (Mombaerts, 2004). Major scientific advances have also been made in the field of nasal toxicology which has grown exponentially over the past two decades since the first published report that long-term inhalation exposures to high concentrations of formaldehyde induce nasal tumors in laboratory rodents (Swenberg et al., 1980; Kerns et al., 1983). The principal aim of the present

article is to provide a brief review of 1) nasal anatomy and physiology of common laboratory animals, 2) the cellular structure and function of the surface epithelial cells that line the nasal passages in these mammalian species, and 3) the adverse effects and remodeling that may occur in the nasal epithelium after short- or long-exposures to various inhaled xenobiotic agents, like those found in environmental and occupational settings. Experimental methods used in toxicologic pathology to assess chemical-induced nasal injury in laboratory animals, and how this animal data may be used to predict the potential risk to human health will also be briefly discussed. The reader is also referred to several other excellent and more comprehensive reviews that have been written on more specific topics in nasal toxicology and pathobiology (Morgan, 1994; Miller, 1995; Harkema, 1996; Herbert, 1999; Ding, 2003; Hastings, 2003; Shusterman, 2003).

The nose is a structurally and functionally complex organ in the upper respiratory tract. It is the primary site of entry for inhaled air in the respiratory system of mammals and therefore has many important and diverse functions. The nose not only serves as the principal organ for the sense of smell, but it also functions to efficiently filter, warm, and humidify the inhaled air before it enters the more delicate distal tracheobronchial airways and alveolar parenchyma of the lung (Cole, 1993). The nasal passages have been described as an efficient “scrubbing tower” for the respiratory tract because it

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effectively absorbs water-soluble and reactive gases and vapors, traps inhaled particles, and metabolizes airborne xenobiotics (Brain, 1970). With its role as an "air conditioner" and a "defender" of the lower respiratory tract, the nose may also be vulnerable to acute or chronic injury caused by exposure to high ambient concentrations of airborne toxic or infectious agents.

The nasal airways and associated paranasal sinuses may be afflicted by many diseases (Goldman et al., 1987; Lund and Maran, 1990; Naclerio and Solomon, 1997). The majority of these conditions are a consequence of microbial infections, allergic reactions, or natural aging. Exposure of humans to toxic agents may also cause or exacerbate nasal disease. In the last 20 years, there has been a marked increase in the study of nasal toxicology and in assessing human risk to nasal injury from inhaled toxicants (Miller, 1995). The reason for this research and related risk assessment efforts is based on the recognition that a wide range of xenobiotics in the air could potentially cause nasal responses that would compromise human health. Environmental exposures of human populations to urban air pollution has been associated with disturbing and deleterious hyperplastic, dysplastic, and inflammatory nasal lesions (Calderon-Garciduenas and Roy-Ocotla 1993; Calderon-Garciduenas et al., 1992, 1994, 1995, 1998, 1999; 2001a, 2001b) and nasal septal necrosis with perforations has been associated with chronic abuse of cocaine and with occupational exposures to chromic acid fumes (Baruthio, 1992; Dingle, 1992). Though nasal cancer is rare in most human populations, nasal carcinomas have been found in individuals repeatedly exposed to nickel dust, ionizing radiation, wood dust, formaldehyde and leather dust (Boysen et al., 1984; Leopold, 1992; Luce et al., 1993). Concern for human risk is also based on the growing number of chemicals found to cause nasal lesions in laboratory animals, such as the nasal cancers induced in rats after long-term, high-level inhalation exposures to formaldehyde (Swenberg et al., 1980; Kerns et al., 1983) and to cigarette smoke (Mauderly et al., 2004).

Though the nasal airways in laboratory animals are recognized as a principal site of injury from experimental exposure to certain inhaled toxicants, extrapolation to humans is complicated by the complexity and diversity of this upper airway among animal species. Responsible estimates of the risks of nasal toxicants to human health must be made with a working knowledge of the differences and similarities in the structure and function of human nasal airways compared to those in the nasal airways of laboratory animals. Comparative aspects of the mammalian nose that have special relevance to inhalation toxicology are highlighted in this brief review along with selected examples of nasal histopathology in laboratory animals exposed by inhalation to controlled concentrations of airborne toxicants. Discussion will be restricted for the most part to toxicant-induced non-neoplastic lesions in the nasal epithelium (e.g., cellular degeneration, cell death, epithelial hyperplasia, metaplasia, and atrophy) and the possible pathophysiologic consequences.

NASAL STRUCTURE AND FUNCTION

Gross Anatomy of the Nose

The nasal airway is divided into two passages by the nasal septum. Each nasal passage extends from the nostrils to the

nasopharynx. The nasopharynx is defined as the airway posterior to the termination of the nasal septum and proximal to the termination of the soft palate. Inhaled air flows through the nostril openings, or nares, into the vestibule, which is a slight dilatation just inside the nares and before the main chamber of the nose. Unlike the more distal main nasal chamber that is surrounded by bone, the nasal vestibule is surrounded primarily by more flexible cartilage. The luminal surface is lined by a squamous epithelium similar to that of external skin. In humans, unlike laboratory animals, the nasal vestibule also contains varying numbers of hairs near the nares. After passing through the nasal vestibule, inhaled air courses through the narrowest part of the entire respiratory tract, the nasal valve (ostium internum), into the main nasal chamber.

Each nasal passage of the main chamber is defined by a lateral wall, a septal wall, a roof, and a floor. The lumen of the main chamber is lined by well-vascularized and innervated mucous membranes that are covered by a continuous layer of mucus. The nasal mucous layer is moved distally, by underlying cilia to the oropharynx where it is swallowed into the esophagus.

Turbinates, bony structures lined by the well-vascularized mucosal tissue, project into the airway lumen from the lateral walls into the main chamber of the nose. Nasal turbinates increase the inner surface area of the nose, which is important in the filtering, humidification, and warming of the inspired air. Though the turbinated, main chamber of the human nose is only about 5 to 8 cm long, the surface area is approximately 150–200 cm², about four times that of the trachea (Guilmette, 1989).

Though there are some general similarities in the nasal passages of most mammalian species, there are also striking interspecies differences in nasal architecture (Figure 1). From a comparative viewpoint, humans have relatively simple noses with breathing as the primary function (microsmatic), while other mammals have more complex noses with olfaction as the primary function (macrosmatic). In addition, the nasal and oral cavities of humans (and some nonhuman primates) are arranged in a manner to allow for both nasal and oral breathing. Most laboratory rodents used in inhalation toxicology studies (e.g., rats, mice, hamsters, guinea pigs) are obligate nose breathers, due to the close apposition of the epiglottis to the soft palate. Interspecies variability in nasal gross anatomy has been emphasized in previous reviews (Negus, 1958; Harkema, 1991) and demonstrated in early studies using silicone rubber casts of the nasal airways (Schneider and Raabe, 1981). Marked differences in airflow patterns among mammalian species are primarily due to variation in the shape of nasal turbinates. The human nose has three turbinates: the superior, middle, and inferior. These structures are relatively simple in shape compared to turbinates in most nonprimate laboratory animal species (e.g., dog, rat, mouse, rabbit) that have complex folding and branching patterns. In laboratory rodents, evolutionary pressures concerned chiefly with olfactory function and dentition have defined the shape of the turbinates and the type and distribution of the cells lining the turbinates. In the proximal nasal airway, the complex maxilloturbinates of small laboratory rodents and rabbits provide far better protection of the lower respiratory tract, by better filtration, absorption, and disposal of airborne particles and gases, than do the simple middle and inferior turbinates

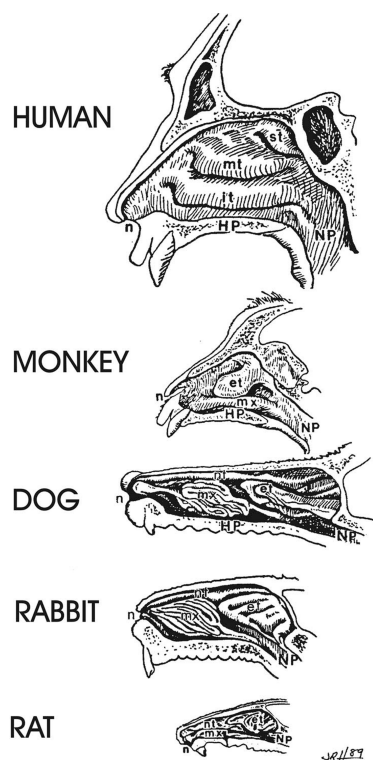


FIGURE 1.—Diagrammatic representation of the exposed mucosal surface of the lateral wall and turbinates in the nasal airway of the human, monkey, dog, rabbit, and rat. The nasal septum has been removed to expose the nasal passage. HP = hard palate; n = naris; NP = nasopharynx; et = ethmoturbinates; nt = nasoturbinate; mx = maxilloturbinate; mt = middle turbinate; it = inferior turbinate; st = superior turbinate.

of the human nose. The highly complex shape of the ethmoturbinates, lined predominantly by olfactory neuroepithelium, in the distal half of the nasal cavity of small laboratory animals is suitably designed for acute olfaction. Differences in the complexity of the gross turbinate structure throughout the nasal airway of the adult laboratory mouse are illustrated in Figure 2.

The Nasal Mucosa and Mucociliary Apparatus

The mucous membranes, or mucosa, lining the nasal airways consist of two layers: the luminal surface epithelium and the underlying connective tissue or lamina propria. The latter layer contains various types and amounts (depending on the intranasal location) of blood and lymphatic vessels, nerves, glands and mesenchymal cells (e.g., fibroblasts, lymphocytes, mast cells), that are embedded in a connective tissue matrix. This review article will focus mainly on the normal cellular composition of the surface epithelial layer and the changes in its cell populations in response to exposures to inhaled toxicants.

Most of the luminal surfaces of the nasal mucosa (with the exception of the most proximal regions of the nasal vestibule) are covered by a watery, sticky material called mucus. Its physical and chemical properties are well suited for its role as an upper airway defense mechanism, filtering the inhaled air by trapping inhaled particles and certain gases or vapors. The mucus is produced by mucous (goblet) cells in the surface

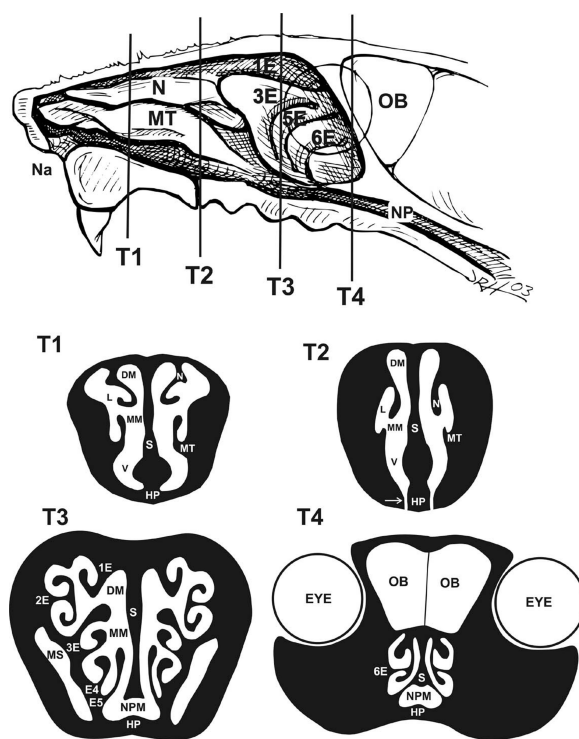


FIGURE 2.—(A) Illustration of the lateral wall and turbinates in the nasal passage of a mouse. The septum has been removed to expose the nasal passage. Vertical lines indicate the location of the anterior faces of 4 tissue blocks routinely sampled for light microscopic examination (T1-T4). (B) Anterior faces of selected tissue blocks from the proximal (T1) to the distal (T4) nasal airway. N = nasoturbinate; MT = maxilloturbinate; 1E-6E = 6 ethmoturbinates; Na = naris; NP = Nasopharynx; HP = hard palate; OB = olfactory bulb of the brain; S = septum; V = ventral meatus; MM = middle meatus; L = lateral meatus; DM = dorsomedial meatus; arrow in T2 = nasopalatine duct; MS = maxillary sinus; NPM = nasopharyngeal meatus.

epithelium and subepithelial glands in the lamina propria. The synchronized beating of surface cilia propels the mucus at different speeds and directions depending on the intranasal location. Mucus covering the olfactory mucosa moves very slowly, with a turnover time of probably several days. In contrast, the mucus covering the transitional and respiratory epithelium is driven along rapidly (1 to 30 mm/min) by synchronized beating of the surface cilia with an estimated turnover time of about 10 minutes in the rat (Morgan et al., 1984). The mucus with the entrapped materials ultimately is propelled by the beating cilia to the naso- and oro-pharynx, and then is swallowed into the esophagus and cleared through the digestive tract. The nasal mucociliary apparatus (i.e., mucus and cilia) exhibits a range of responses to inhaled xenobiotic agents and can be a sensitive indicator of toxicity (Morgan et al., 1986b). Since this upper airway apparatus is one of the first lines of defense against inhaled pathogens, dusts, and irritant gases, toxicant-induced compromises in its defense capabilities could lead to increased nasal infections and increased susceptibility to lower respiratory tract diseases.

The amount of intraepithelial mucosubstances (i.e., stored mucous product within mucous secretory cells present in the surface epithelium) in the nasal airways of macaque monkeys has been estimated using histochemical and morphometric

techniques (Harkema et al., 1987a). Like the anterior-posterior gradient increase of mucous cells in the human nasal airway (Morgensen and Tos, 1977), there is an anterior-posterior gradient increase in the amount of mucosubstances in the nasal cavity of these monkeys. Scant amounts of both neutral and acidic mucosubstances are also present in the anterior nasal airway, while the respiratory epithelium covering the maxilloturbinate and nasopharynx has copious amounts of stored secretory product.

Similar estimates of intraepithelial mucosubstances in the anterior nasal airway and nasopharynx have been made for the F344 rat (Harkema et al., 1989). In contrast to the monkey, the rat has considerably more intraepithelial mucosubstances in the anterior septal respiratory epithelium, than it does in the more distal respiratory epithelium lining the nasopharynx. Like monkeys, however, laboratory rats normally contain very little mucosubstances in the transitional epithelium lining the lateral wall of their proximal nasal passages.

Since mucus is a protective lining substance for upper airway epithelium, intranasal regional differences in intraepithelial mucosubstances may be useful in predicting sites of certain toxicant-induced nasal injury. For example, mucus is known to be a strong anti-oxidant agent (Cross et al., 1984), and inhalation of ambient concentrations of ozone, a strong oxidant in urban smog, has been reported to injure regions of both the monkey and rat nasal airways that contain very little intraepithelial mucosubstances, and spare adjacent regions that contain abundant stored secretory product (Harkema et al., 1987b, 1987c, 1989, 1997, 1999; Johnson et al., 1990; Hotchkiss, 1994). More studies designed to examine the protective effects of mucus in upper airways are needed to understand the pathogenesis of oxidant-induced injury, or other toxicant-induced injury, to nasal epithelium and its relationship to the nasal mucociliary apparatus.

Cellular Composition of Nasal Surface Epithelium

Besides the differences in the gross architecture of the nose among different mammalian species, there are also species differences in the surface epithelial cell populations lining the nasal passages. These differences among species are found in the distribution of nasal epithelial populations and in the types of nasal cells within these populations. There are, however, four distinct nasal epithelial populations in most animal species. These include the squamous epithelium, which is primarily restricted to the nasal vestibule; ciliated pseudostratified cuboidal/columnar epithelium, or respiratory epithelium, in the main chamber and nasopharynx; nonciliated cuboidal/columnar epithelium, or often termed transitional epithelium, lying between squamous epithelium and the respiratory epithelium in the proximal or anterior aspect of the main chamber; and olfactory epithelium, located in the dorsal or dorsoposterior aspect of the nasal cavity. Figure 3 illustrates the general distribution of these distinct epithelial cell populations in the nasal cavity of the laboratory rat and monkey. The reader is referred to other reports for a more thorough and detailed description of the intranasal distribution of airway surface epithelia in laboratory rodents and non-human primates (Mery et al., 1994a; Kepler, 1995).

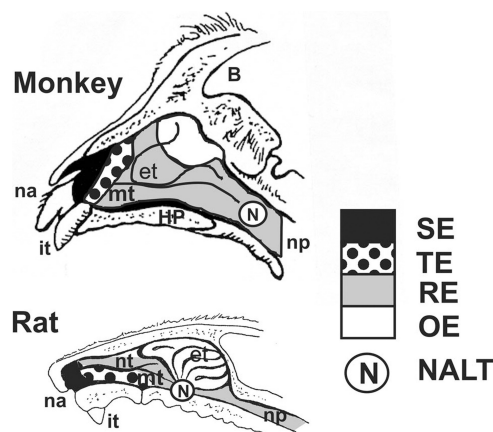


FIGURE 3.—Distribution of the surface epithelia lining the nasal lateral wall of the monkey and rat. Four distinct epithelial cell populations line both mammalian species—SE = squamous epithelium; TE = transitional epithelium; RE = respiratory epithelium; OE = olfactory epithelium. However, considerably more olfactory epithelium (OE) lines the intranasal surface of the rat compared to the monkey. NALT = Nasal associated lymphoid tissue; et = ethmoturbinate; mt = maxilloturbinate; nt = nasoturbinate; na = nasus; it = incisor tooth; B = brain.

Olfactory Epithelium

The major difference in nasal epithelium among animal species is the percentage of the nasal airway that is covered by olfactory epithelium (OE). For example, the OE covers a much greater percentage of the nasal cavity in rodents, which have an acute sense of smell, as compared to monkeys or humans, whose sense of smell is not as well developed. Gross et al. morphometrically determined that approximately 50% of the nasal cavity surface area in F344 rats is lined by this sensory neuroepithelium (Gross et al., 1982). OE in humans is limited to an area of about 500 mm², which is only 3% of the total surface area of the nasal cavity (Sorokin, 1988). Mice, rabbits, and dogs are much closer to rats than humans or monkeys in respect to the relative amount of OE within their nasal passages.

Three epithelial cell types compose the OE. These are the olfactory sensory neuron (OSN), the supporting (sustentacular) cell, and the basal cell (Figure 4A). The OSNs are bipolar neuronal cells interposed between the sustentacular cells (Vollrath et al., 1985; Farbman, 1994). The dendritic portions of these neurons extend above the epithelial surface and terminate into a bulbous olfactory knob from which protrude on average 10–15 immotile cilia (Menco, 1983). These cilia, about 50 microns in length and 0.1–0.3 microns in diameter, are enmeshed with each other and with microvilli in the surface fluid, and provide an extensive surface area for reception of odorants. Menco has estimated that the ciliary membranes increase the receptive surface of the OSN by 25–40-fold (Menco, 1980).

It should be emphasized that the ciliary membranes of the OSN contain the odorant receptors (ORs) responsible for the chemical interaction with and initial detection of inhaled odors. ORs are G protein-coupled, seven transmembrane membrane proteins that are encoded by the largest gene families known to exist in a given animal genome (Mombaerts, 1999b, 2001). Odorant genes were discovered by Linda Buck

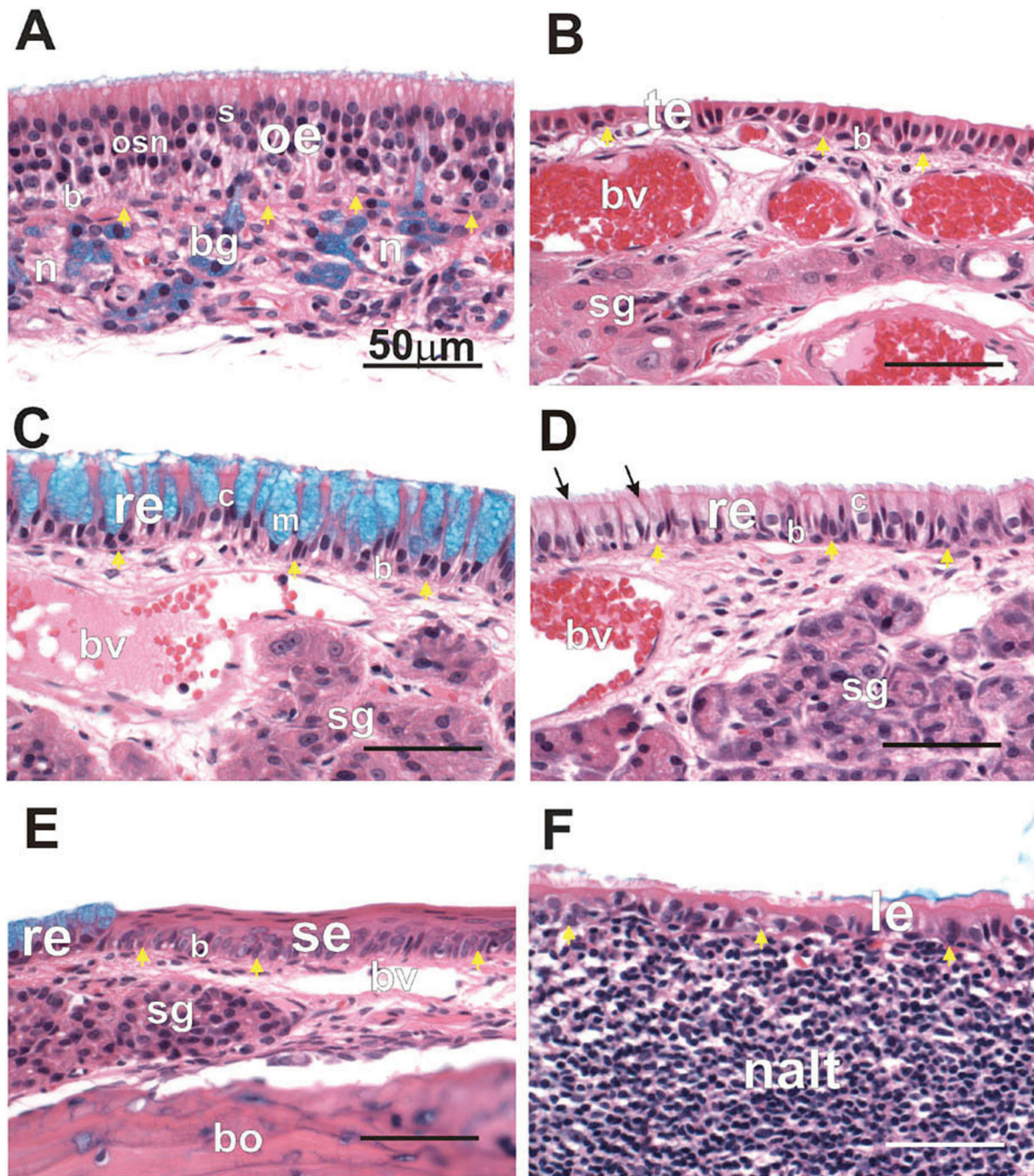


FIGURE 4.—Light photomicrographs of the different types of surface epithelia that line the rat nasal airways. Tissue sections are stained with hematoxylin and eosin for routine cellular and acellular morphology, and alcian blue (AB; pH 2.4) to identify epithelial cells with acidic mucosubstances (i.e., mucous cells). (A) Olfactory epithelium (oe) lining the dorsal septum from tissue section T3 and containing a prominent apical row of nuclei and cytoplasm of sustentacular cells (s), several middle layers of nuclei in the cell bodies of olfactory sensory neurons (OSN), and basal cells (b) lining the basal lamina. The luminal surface contains numerous cilia from OSNs and microvilli projecting from the apical surface of sustentacular cells. Bowman's glands (bg), with large amounts of intracellular AB-stained mucosubstances, and nerve bundles (n) are present in the lamina propria. Yellow arrowheads identify the basal lamina separating the surface epithelium from the underlying lamina propria. (B) Transitional epithelium (te) lining the proximal lateral meatus in section T1 and composed of nonciliated, cuboidal to low columnar apical cells, and basal cells (b). Blood vessels (bv) and subepithelial glands (sg) are present in the lamina propria. (C) Respiratory epithelium (re) lining the proximal septum in section T1 containing columnar, ciliated cells (c), mucous (goblet) cells (m) with large amounts of AB-stained mucosubstances, and basal cells (b). (D) Respiratory epithelium lining the midseptum from the middle aspect of the nasal airway in section T2 and containing ciliated cells (c), basal cells (b), and narrow, nonciliated serous cells (arrows), interspersed among the ciliated cells, that normally contain no or scant amounts of acidic mucosubstances; (E) Stratified squamous epithelium (se) lining the floor of the ventral meatus in the proximal nasal airway (T1). There is a sharp transition from respiratory epithelium (re) containing numerous AB-staining mucous cells to se. A thin lamina propria containing some subepithelial glands (sg) and blood vessels (bv) lies between the surface epithelium and bone (bo). (F) Lymphoepithelium (le) containing both ciliated and nonciliated cells, along with some intraepithelial lymphocytic cells, overlie the nasal associated lymphoid tissue (NALT) located in the floor of the nasal airway at the opening of the nasopharyngeal meatus (T3).

and Richard Axel who were awarded the 2005 Nobel Prize in physiology or medicine for their landmark work in the cellular and molecular biology of olfaction (Buck and Axel, 1991). Their elegant and novel work was one of the first applications of degenerate polymerase chain reaction (PCR). It is now estimated that there are 500–1000 OR genes in the rat and mouse (Buck, 1992), and ~1000 sequences in humans, residing in multiple clusters spread throughout the genome, with more than half being pseudogenes (Mombaerts, 2001).

A single OR gene is expressed in a minute subset of OSN with the current belief that each OSN expresses only a single OR (1 receptor-1 neuron rule). Interestingly, rat and mouse OR genes are expressed in OSNs within one of four, even-sized, distinct topographical zones in the OE lining the nasal cavity (Vassar et al., 1993; Ressler et al., 1994). OSNs expressing a given OR are distributed in a random, punctate, manner within a zone. With the nasal cavity of a mouse, there are approximately 2 million OSNs.

The axon of the OSN originates from the base of the cell and passes through the basal lamina to join axons from other OSNs forming nonmyelinated nerve fascicles, or bundles, in the lamina propria. These olfactory nerves perforate the bony cribriform plate, that separates the nasal cavity from the brain, and form the outer olfactory nerve layer of the olfactory bulb. Axons of OSNs that express the same OR gene converge with extreme precision on ~2000 signal-processing modules called “glomeruli” that reside in distinct locations within olfactory bulb (Vassar et al., 1994; Mori et al., 1999). Glomeruli are relatively large spherical neuropils (100–200 microns in diameter) in which the axons of OSNs form synaptic connections on the dendrites of mitral and tufted cells, the output neurons of the olfactory bulb (Mori et al., 2000; Nagayama et al., 2004). Transmission of olfactory information is further sent through the axons of the mitral and tufted cells to the olfactory cortex.

Because the OE is in direct contact with the environment, inhaled xenobiotic agents, such as airborne chemical toxicants or infectious microbial agents, may induce cell injury and death of OSNs. Unlike other neurons in the body, the OSNs are able to regenerate when there is neuronal cell loss and there is continual neurogenesis in this nasal epithelium to maintain its olfactory function. Initial studies suggested that OSNs have a steady 28- to 30-day turnover rate in the rat (Graziadei and Monti-Graziadei, 1977, 1978). Others have more recently shown that many OSNs are more long-lived despite continuous neurogenesis of the OE (Hinds et al., 1984; Mackay-Sim and Kittel, 1991). The constant turnover of OSNs is due to the capacity of progenitor cells in the basal cell layer of the OE to proliferate and differentiate into mature OSNs (Mackay-Sim and Kittel, 1991; Huard and Schwob, 1995; Schwob et al., 1995; Huard et al., 1998; Jang et al., 2003; Schwob, 2005).

The rate of basal cell proliferation is markedly increased with experimental induction of OSN injury and death whether that be through surgical bullectomy (Kastner et al., 2000) or axotomy (Suzuki and Takeda, 1991), or intranasal exposure to some chemical toxicants such as zinc sulfate (Margolis et al., 1974; McBride et al., 2003). The unique ability of OSNs to regenerate makes the OE an excellent model tissue to study the underlying cellular and molecular mechanisms of neurogenesis and axon regeneration (Graziadei and Monti-

Graziadei, 1978). Though the process of neurogenesis and regeneration of the OE is still poorly defined, recent studies of olfactory mucosal injury and repair suggest that inflammatory signaling pathways may play a key role in the regulation of OSN regeneration (Giannetti et al., 1995; Nan et al., 2001; Getchell et al., 2002; Bauer et al., 2003).

The olfactory epithelium also contains two types of basal cells—horizontal (HBC) and globose (GBC). HBCs are thin cells located along the basal lamina and share many of the same morphologic and histochemical features as the basal cells of nasal respiratory epithelium (e.g., contain keratins). In contrast GBCs are morphologically more round or oval and are located above the HBCs. These cells have a more electron-lucent cytoplasm than the HBCs and are not immunohistochemically reactive for keratin. Some of the GBCs are the progenitor cells for OSNs, while the HBCs give rise to GBCs (Caggiano et al., 1994; Goldstein and Schwob, 1996). Multipotent basal cells within the OE or in Bowman's gland ducts are the likely progenitors for sustentacular cells that are described below (Huard et al., 1998; Weiler and Farbman, 1998).

Sustentacular (or supporting) cells are columnar epithelial cells that span the entire thickness of the OE from the airway surface to the basal lamina. The distinct oval nuclei of the sustentacular cells are aligned in a single row along the apical aspect of the OE and are the most apically located epithelial nuclei within the mammalian OE (Figure 4A). The supranuclear portion of the cell is broad while the portion of the cell below the nucleus tapers to a thin foot-like process that attaches to the basal lamina. These supporting cells surround the OSNs making multiple contacts with OSNs through fine cellular extensions (Breipohl et al., 1974; Morrison and Costanzo, 1990). The apical surfaces of sustentacular cells are lined by numerous long microvilli that intermingle with the thin cilia of the OSNs along the surface of the airway lumen. The supranuclear cytoplasm of sustentacular cells has abundant smooth endoplasmic reticulum and xenobiotic-metabolizing enzymes (e.g., cytochrome P-450, flavin-containing monooxygenases, N-acetyltransferases). The metabolism in these cells may be important in detoxification of inhaled xenobiotics and in the function of smell (Dahl and Hadley, 1991; Reed, 1993; Ding, 2003; Genter, 2004; Ling et al., 2004). Sustentacular cells are also thought to contribute to the regulation of the ionic composition of the overlying mucous layer which undoubtedly affects the chemical interactions between odors and their ORs. The microvilli of these cells contain amiloride-sensitive sodium channels (Menco et al., 1998), while the lateral surfaces contain a water channel, aquaporin Type 3 (Verkman, 2000). Mammalian sustentacular cells do not contain mucin glycoproteins characteristic of the columnar mucus-secreting epithelial cells in nasal respiratory epithelium (e.g., mucous goblet cells). The production and secretion of mucus covering the luminal surface of the OE is restricted to the subepithelial Bowman's Glands that are described next.

Besides the principal epithelial cells of the OE that include the sustentacular cells, OSNs, and basal cells, there are at least five other morphologically distinct but much less abundant epithelial cells in the OE that have been reported in the literature. Collectively these cells have been termed as microvillous cells because of their distinct luminal surface that

is lined by numerous microvilli (Menco, 2003). Though these apically located and widely scattered cells have specific morphologic or immunohistochemical features that distinguish them from sustentacular cells (another cell with a distinct microvillar apical surface), the exact function of these microvillous cells have not yet been determined.

Bowman's Glands, located in the underlying lamina propria and interspersed among the olfactory nerve bundles, are simple tubular-type glands composed of small compact acini. Ducts from these glands transverse the basal lamina at regular intervals and extend through the OE to the luminal surface. Bowman's glands contain copious amounts of neutral and acidic mucosubstances that contribute to the mucous layer covering the luminal surface of the olfactory epithelium (Figure 4A). Like the sustentacular cells, both the acinar and duct cells of Bowman's glands contain many xenobiotic-metabolizing enzymes.

Squamous Epithelium

The nasal vestibule is completely lined by a lightly keratinized, stratified squamous epithelium. It is composed of basal cells along the basal lamina and several layers of squamous cells, which become progressively flatter toward the luminal surface of the airway (Figure 4E). Only 3.5% of the entire nasal cavity of the F344 rat is lined by squamous epithelium. This region of the nasal mucosa probably functions like the epidermis in the skin, to protect the underlying tissues from potentially harmful atmospheric agents.

Transitional Epithelium

Distal to the stratified squamous epithelium and proximal to the ciliated respiratory epithelium is a narrow zone of nonciliated, microvilli-covered surface epithelium, which has been referred to as nasal, nonciliated, respiratory epithelium or nasal transitional epithelium (Figure 4B). Common, distinctive features of this nasal epithelium in all laboratory animal species and humans include: (1) anatomical location in the proximal aspect of the nasal cavity between the squamous epithelium and the respiratory epithelium; (2) the presence of nonciliated cuboidal or columnar surface cells and basal cells; (3) a scarcity of mucous (goblet) cells and a paucity of intraepithelial mucosubstances; and (4) an abrupt morphological border with squamous epithelium, but a less abrupt border with respiratory epithelium.

In rodents, this surface epithelium is thin (i.e., 1 to 2 cells thick), pseudostratified, and composed of 3 distinct cell types (basal, cuboidal, and columnar) (Monteiro-Riviere and Popp, 1984). In contrast, transitional epithelium in monkeys is thick (i.e., 4 to 5 cells), stratified, and composed of at least 5 different cell types (Harkema et al., 1987d). The luminal surfaces of transitional epithelial cells lining the nasal airway possess numerous microvilli. Luminal, nonciliated cells in the transitional epithelium of rodents have no secretory granules but do have abundant SER in their apices (Harkema et al., 1987d). SER is an important intracellular site for xenobiotic metabolizing-enzymes, including cytochromes P-450. The prominent presence of SER in these cells, like the sustentacular cells in the OE, suggests that they may have roles in the metabolism of certain inhaled xenobiotics.

Respiratory Epithelium

The majority of the nonolfactory nasal epithelium of laboratory animals and humans is ciliated respiratory epithelium (Figure 4C, D). Approximately 46% of the nasal cavity in a F344 rat is lined by respiratory epithelium (Gross et al., 1982). Although this pseudostratified nasal epithelium is similar to ciliated epithelium lining other proximal airways (i.e., trachea and bronchi), it also has unique features. Nasal respiratory epithelium in the rat is composed of six morphologically distinct cell types: mucous, ciliated, nonciliated columnar, cuboidal, brush, and basal (Monteiro Rivier and Popp, 1984). These cells are unevenly distributed along the rat mucosal surface. Using scanning electron microscopy, Popp and Martin demonstrated a proximal-to-distal increase in ciliated cells along the lateral walls of the rat. In the nasal septum of the rat, ciliated cells are evenly distributed from proximal to distal sites.

Like the ciliated cell, the mucous cell is unevenly distributed in the respiratory epithelium of the nasal cavity. In the normal rat, mucous cells are predominantly located in respiratory epithelium lining the proximal septum and the nasopharynx (Harkema et al., 1989). Serous cells are the primary secretory cells in the remainder of the respiratory epithelium in rodents. Interestingly, secretory cells in the respiratory epithelium of both rats (Yamamoto and Masuda, 1982) and mice (Matulionis and Parks, 1973) have abundant SER. This suggests that these cells, like the nonciliated cell in the transitional epithelium, may have metabolic capacities for certain xenobiotic agents. Research in the area of xenobiotic metabolism in nasal respiratory epithelium, like the OE, has demonstrated the presence of many enzymes previously described in other tissues (Bogdanffy et al., 1987; Bogdanffy, 1990; Keller et al., 1990). In particular, carboxylesterase, aldehyde dehydrogenase, cytochrome P-450, epoxide hydrolase, and glutathione S-transferases have been localized by histochemical techniques. The distribution of these enzymes appears to be cell-type-specific, and the presence of the enzyme may predispose particular cell types to enhanced susceptibility or resistance to chemical-induced injury.

Lymphoepithelium and NALT

In addition to the four principal nasal epithelia already described, there is another specialized epithelium, i.e., lymphoepithelium, in animal nasal airway that covers discrete focal aggregates of nasal-associated lymphoid tissue (NALT) in the underlying lamina propria (Figure 4F). In rodents, NALT with associated LE is restricted to the ventral aspects of the lateral walls at the opening of the nasopharyngeal duct (Belai et al., 1977; Reuman et al., 1989; Spit et al., 1989; Kuper et al., 1990; Ichimiya et al., 1991; Asanuma et al., 1995; Heritage et al., 1997). The overlying LE is composed of cuboidal ciliated cells, a few mucous cells, and numerous nonciliated, cuboidal cells with luminal microvilli (so-called membranous or M cells) similar to those in the gut- and bronchus-associated lymphoid tissues (GALT and BALT, in the intestinal and lower respiratory tracts, respectively). M cells are thought to be involved in the uptake and translocation of inhaled antigen from the nasal lumen to the underlying lymphoid structures.

NALT, with its specialized lymphoepithelium, has also been described in the nasopharyngeal airways of the monkey, but these lymphoid structures LE are more numerous and are located on both the lateral and septal walls of the proximal nasopharynx (Loo and Chin, 1974; Harkema et al., 1987d). The correlate of NALT in humans is Waldeyer's ring, the oropharyngeal lymphoid tissues composed of the adenoid, and the bilateral tubule, palatine, and lingual tonsils (Brandtzaeg, 1984).

The location of NALT at the entrance of the nasopharyngeal duct is a very strategic position as most of the nasal secretions and inhaled air, both presumably laden with antigenic material, pass over this area. Though the function of NALT and its place in the general mucosal-associated lymphoid system are not fully understood, these mucosal lymphoid tissues may have an important function in regional immune defense of the upper airways. NALT has been studied primarily in rat and mouse models (Belai et al., 1977; Reuman, 1989; Spit et al., 1989; Kuper et al., 1990; Ichimiya et al., 1991; Koornstra et al., 1993; Asanuma et al., 1995; Heritage et al., 1997). Immunohistochemical characterization of rat NALT has demonstrated that B- and T-cells are distributed in distinct areas with a high CD4:CD8 T-cell ratio and a predominance of B- over T-cells (Koornstra et al., 1993). Initial studies in mice suggest the NALT is distinct from that found in rats and, if examined solely on immune cell content and subset ratios, more closely resembles the spleen and not the Peyer's Patches located in the intestinal mucosa (Heritage et al., 1997). However, the capability of NALT to elicit specific IgA responses locally suggests that this structure might represent a unique mucosal lymphoid tissue that is capable of expressing both mucosal and systemic immune responses.

Though it is clear that NALT plays a key role in nasal mucosal immunity, the toxicity to NALT by inhaled toxicants has unfortunately not been the focus of specific investigation. It has been recently recommended that more research efforts be made in this area and that the histopathological examination of NALT be routinely included in standard guideline-driven inhalation toxicity studies (Kuper et al., 2003).

NASAL EPITHELIAL INJURY AND REMODELING INDUCED BY INHALED TOXICANTS

Regional Dosimetry and Lesion Distribution

Laboratory rodents are susceptible to injury from a wide range of xenobiotic agents such as formaldehyde, chlorine, ozone, methyl bromide, naphthalene, propylene oxide, hydrogen sulfide and cigarette smoke (Walker, 1983; Buckley et al., 1984; Harkema et al., 1989; Johnson et al., 1990; Monticello et al., 1990; Brennen et al., 2000a, 2002; Rios-Blanco et al., 2003; Phimister et al., 2004). Chemical toxicants may induce nasal lesions when delivered by inhalation (Buckley et al., 1984; Barrow, 1986; Harkema, 1990; Uraih et al., 1990) or noninhalation (Reznik-Schuller, 1983; Barrow, 1986; Brittebo et al., 1991; Genter et al., 1992) routes of exposure. These nasal lesions include both nonneoplastic (e.g., inflammation, epithelial cell necrosis, epithelial hyperplasia/metaplasia) and neoplastic (e.g., squamous cell carcinoma) changes. It is not the intent in this chapter to provide a complete overview of all chemical-induced lesions that may be encountered in the nasal cavity. A small selection

of examples will be discussed below along with the possible factors involved in the pathogenesis of these alterations. The reader is referred to the references that describe in detail the histologic characteristics of various toxicant-induced lesions, which include hyperplasia in squamous epithelium (glutaraldehyde), mucous cell metaplasia in transitional epithelium (ozone or chlorine), squamous metaplasia in transitional and respiratory epithelium (formaldehyde and cigarette smoke), intraglandular accumulations of proteinaceous material (dimethylamine), degeneration of olfactory epithelium (dibasic esters), and many other changes (e.g., inflammation, ulceration, bony atrophy).

Toxicant-induced nasal lesions in laboratory animals generally exhibit characteristic, site-specific, distribution patterns (Morgan et al., 1986a; Morgan and Monticello, 1990; Morgan, 1994, 1997). For example, formaldehyde induces lesions in rats that are essentially confined to the anterior nose, in regions lined by transitional and respiratory epithelium. In contrast, the nasal damage induced by methyl bromide is confined to the olfactory epithelium, and the transitional and respiratory epithelia are not affected. Each nasal toxicant appears to exhibit its own characteristic pattern of lesion distribution. Site specificity of nasal lesions have been reported for each of the four principal epithelial types lining the nasal airways of rodents, including the squamous (Gross et al., 1994), transitional (Harkema et al., 1994), respiratory (Morgan et al., 1986b) and olfactory (Genter et al., 1992) regions. Site-specific lesions have been described in detail for ozone (Harkema et al., 1994) and formaldehyde (Monticello et al., 1989, 1991) in both laboratory rodents and nonhuman primates.

Determination of the precise location of the induced lesions in the nose of the laboratory animal is the first step in understanding the critical factors involved in the pathogenesis of the injury. Therefore, it is important to use a consistent method of analyzing and recording lesion distribution in the complex nasal airways of laboratory rodents. Young provided the first description of a systematic approach to sectioning the nose of rats for histologic analysis (Young, 1981). Mery et al. (1994a) provided a series of diagrams of the nasal passages of the F344 rat and B6C3F1 mouse, designed for mapping nasal lesions in toxicological studies. Similar diagrams have been provided for the rhesus monkey (Kepler, 1995). Morgan has emphasized the usefulness and importance of using this approach for recording nasal lesions identified by light microscopy (Morgan, 1994). Hardisty et al. (1999) used nasal mapping to demonstrate the distribution of olfactory lesions induced by a select group of inhaled toxicants. More recently the lesions induced by beta-beta'-iminodipropionitrile, methyl iodide and methyl methacrylate in the rat nasal cavity have been mapped in three-dimensions to better visualize the intranasal distribution of these alterations and to further understand the possible roles of local bioactivation and dosimetry in the formation of these chemical-induced lesions (Robinson et al., 2003). Nasal mapping, combined with immunohistochemistry, has also been used to illustrate the distribution of endogenous levels of antioxidant compounds in the nasal mucosa of normal laboratory rats (Reed et al., 2003).

Lesion distribution in the nose may be attributed to local dose, regional tissue susceptibility, or a combination of these factors (Morgan, 1994). Mery et al. recorded the distribution

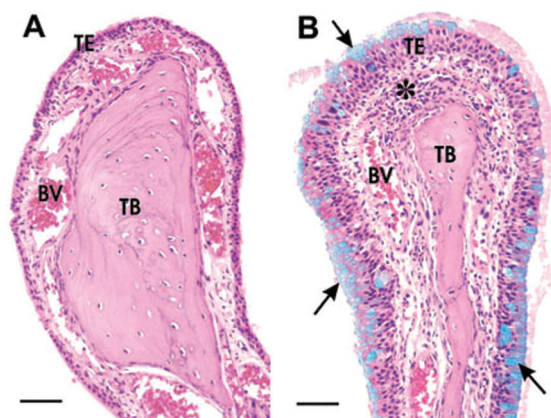


FIGURE 5.—Light photomicrographs of the maxilloturbinate in the proximal nasal airways of rats that were exposed to either filtered air alone (0 ppm ozone) (A) or to a continuous exposure of 0.5 ppm ozone for 13 weeks (B). Tissues were stained with hematoxylin and eosin, and alcian blue (AB; pH 2.4). The transitional epithelium (te) lining the maxilloturbinate in B is markedly thickened due to epithelial hyperplasia and mucous cell metaplasia (arrows identify mucous cells with AB-stained mucosubstances). Numerous inflammatory cells are also present in the underlying lamina propria (asterisk) in B, but not in A. The turbinate bone (tb) is markedly atrophic in the maxilloturbinate of the rat that was exposed to ozone (B) compared to the normal maxilloturbinate in the air-exposed animal (A). Bars = 50 microns.

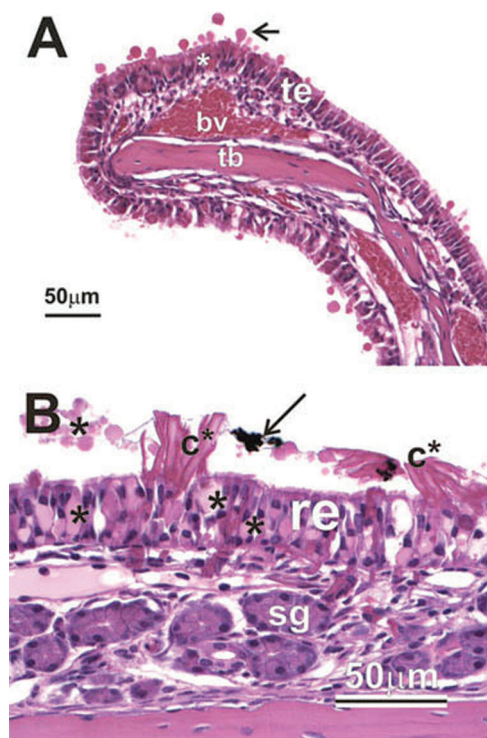


FIGURE 6.—Light photomicrographs of A) transitional epithelium (te) lining the proximal aspect of the maxilloturbinate and B) respiratory epithelium (re) lining the midseptum from mice that were repeatedly exposed to a high airborne concentration of ultrafine carbon black particles. Hyaline proteinaceous material (eosinophilic globules; hyalinosis; asterisks) is present in several nonciliated cells of the te and re. Similar hyaline droplets are being extruded from some of these epithelial cells. Arrows = individual or small aggregates of carbon black particles; n = nerves in lamina propria; g = glands in lamina propria; C = eosinophilic crystals of the proteinaceous material; v = blood vessels in lamina propria; tb = turbinate bone. Tissues stained with hematoxylin and eosin.

of chemical-induced nasal lesions, from previously reported studies, on both transverse and longitudinal diagrams of the nose. These lesions were selected because they occurred in diverse regions of the nose, had characteristic patterns of distribution, and exhibited clear concentration-response relationships. They concluded that some irritants, like formaldehyde, induce lesions in the major inspiratory airways of the nose, especially in the nasal vestibule, in the proximal regions of the lateral and middle medial meatus, and in the dorsal medial meatus. This characteristic pattern is attributed to airflow-driven deposition. Similar factors could account for the proximal-distal severity gradient of nasal lesions induced by many inhaled sensory irritants (Buckley et al., 1984). The pattern of epithelial lesion distribution induced by other xenobiotics, like ozone or chlorine, suggests that tissue or cellular susceptibility in addition to air flow are important in the pathogenesis of the specific lesions caused by these irritating, oxidant gases.

Regional tissue susceptibility is generally a consequence of local metabolism (Vollrath et al., 1985), whereas local dosimetry may be influenced by airflow, mucous flow, blood flow, physicochemical properties of the chemical, or other factors in addition to metabolism (Morgan, 1994). The intranasal metabolism of chlothiamid (Brittebo et al., 1991), acetaminophen (Jeffery and Haschek, 1988), and beta-beta'-iminodipropionitrile (Genter et al., 1992) by cytochromes P-450 is a principal factor for the site-specific nature of the nasal lesions induced by these compounds. In addition, the

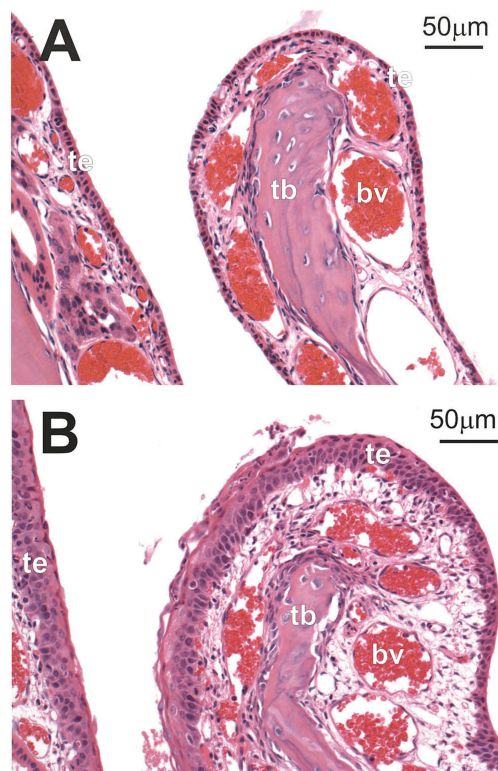


FIGURE 7.—(A) Normal transitional epithelium (te) lining the maxilloturbinate and lateral wall in nasal section T1 of a rat. (B) Squamous metaplasia of the te lining both the maxilloturbinate and lateral wall in nasal section T1 of a rat repeatedly exposed to a high concentration of mainstream cigarette smoke. tb = turbinate bone; bv = blood vessel in the lamina propria.

metabolism of dibasic esters by nasal carboxylesterase activity appears to be involved in the site-specific injury to olfactory epithelial sustentacular cells (Bogdanffy et al., 1987; Trela et al., 1992).

Chemical-Induced Lesions of Squamous and Transitional Epithelium

Though many inhaled nasal toxicants cause lesions in the proximal nasal passages, only a few of these irritants induce structural damage to the squamous epithelium lining the nasal vestibule or the ventral meatus in rodents. Therefore, the squamous epithelium is believed to be more resistant to injury than transitional or respiratory epithelium. A few irritants, like dimethylamine (Buckley et al., 1985), glutaraldehyde (Gross et al., 1994), ammonia (Bolon et al., 1991), and hydrogen chloride (Jiang et al., 1983) do cause lesions to this nasal epithelium. The caustic nature of these chemicals and the air-flow driven, locally high dose to this tissue are the probable reasons for these chemical-induced lesions in the squamous epithelium, rather than cellular susceptibility. Acute alterations of squamous epithelium are usually erosion or ulceration, with or without accompanying inflammation. Lesions induced after long-term exposures may include hyperplasia or hyperkeratosis. These latter changes may represent defensive or adaptive responses to the prolonged exposure to the irritant, and/or early indicators of a subsequent neoplastic response.

The transitional epithelium is thought to be more sensitive than squamous epithelium to certain toxicants. Exposure to less irritating oxidants, like ozone (Harkema, 1994) and chlorine gas (Wolf et al., 1995), causes hyperplastic and metaplastic changes in the transitional epithelium of laboratory animals. These changes are often preceded by acute inflammation with an influx of neutrophils into the lamina propria, luminal epithelium, and airway lumen. Ozone-induced epithelial hyperplasia and mucous cell metaplasia in the transitional epithelium lining the maxilloturbinate of a laboratory rat is illustrated in Figure 5.

Ozone, the principal oxidant air pollutant in photochemical smog, has been shown to cause nasal epithelial and inflammatory responses in laboratory animals and humans (Nikasinovic et al., 2003). This irritating, oxidant gas induces epithelial hyperplasia and mucous cell metaplasia in the transitional epithelium of both rats (Harkema et al., 1989) and monkeys (Harkema et al., 1987b) after short-term exposures (Figure 4). The reason(s) for the rapid induction of mucous cell metaplasia (the histologic appearance of numerous mucous cells in an epithelium normally devoid of these mucus-secreting cells) in transitional epithelium after ozone-exposure is not fully known. Influx of neutrophils, an increase in epithelial DNA synthesis, and an overexpression of a mucin-specific gene (MUC5AC) precedes the onset of mucous cell metaplasia induced by ozone (Cho et al., 1999). Ozone-induced mucous cell metaplasia has been shown to be dependant in part on the influx of neutrophils in the nasal airways (Cho et al., 2000; Luster, 2001; Wagner et al., 2001; Harkema and Wagner, 2002).

Interestingly the nasal inflammatory and proliferative responses to acute, short-term, ozone exposure initially decrease as the mucous cell metaplasia develops in the transitional epithelium, even in the face of continuous acute

exposure. Since airway mucus is a good antioxidant (Cross et al., 1984), it is plausible that these newly formed intraepithelial mucosubstances, with a corresponding increase in secreted mucins, act as a protective shield for the nasal epithelium and underlying lamina propria, preventing further injury by the continued ozone exposure. Therefore, the reduction in surface epithelial injury would lead to subsequent attenuation of the inflammatory response.

However, long-term exposures of F344 rats to ozone (0.5 or 1.0 ppm) for 20 months (6 h/day, 5 days/week) have been shown to cause chronic rhinitis, marked epithelial proliferation with mucous cell metaplasia in the transitional epithelium and an associated reduction in the mucous flow rates along the luminal surfaces in the proximal nasal airways (Harkema et al., 1999; Harkema et al., 1997; Harkema et al., 1994). It has been suggested that these structural and functional alterations could significantly modify an important respiratory defense mechanism of the upper airways (i.e., mucociliary clearance) and leave the more distal pulmonary airways vulnerable to potentially injurious concentrations of inhaled xenobiotics or infectious agents.

Often accompanying epithelial hyperplasia and mucous cell metaplasia in the transitional, and respiratory, epithelium of laboratory rodents repeatedly exposed to chemical irritants, like ozone, is the accumulation of a proteinaceous material in the supra- or sub-nuclear cytoplasm of nonciliated cuboidal/columnar epithelial cells (Boorman et al., 1990; Harkema, 1990; Herbert et al., 1996; Herbert, 1999). With routine histologic, hematoxylin and eosin staining this intracellular accumulation has a homogeneous eosinophilic, hyaline, appearance and has been often referred to as epithelial hyalinosis, hyaline degeneration, eosinophilic globules, or intracytoplasmic hyaline droplets (Figure 6). Ultrastructurally the rough endoplasmic reticulum of the affected epithelial cells is markedly dilated with this proteinaceous material. This epithelial change may also be associated with secreted droplets of this proteinaceous material in the nasal airway lumen and/or the accumulation of eosinophilic crystals either in the airway lumen or within the altered epithelium. This alteration is a commonly observed non-specific epithelial change in the nasal epithelium of both mice and rats (Nagano et al., 1997), but it has also been reported in the nasal transitional and respiratory epithelium of laboratory monkeys exposed to ozone (Harkema et al., 1987c; Nagano et al., 1997). In rodents, intracytoplasmic accumulation of proteinaceous material may also occur in the epithelial cells of glands in the lamina propria below the respiratory epithelium and often at the junction of olfactory and respiratory epithelium in the nasal mucosa of rodents. This epithelial alteration has also been commonly observed in the sustentacular cells of nasal olfactory epithelium of normal aging mice or in younger mice exposed to certain inhaled toxicants (Herbert et al., 1996; Nagano et al., 1997; Herbert, 1999; Katagiri et al., 2000; Giannetti et al., 2004).

Ward et al. recently reported that this intracytoplasmic proteinaceous material in the nasal epithelium of mice contains Ym1/2 chitinase proteins (Ward et al., 2001). Ym proteins (isotypes Ym1 and Ym2) are members of the chitinase gene family and have been identified in various murine tissues, besides the nose, including the lung, oral cavity, esophagus, glandular stomach, bile duct, gall bladder, and in peritoneal

and bronchoalveolar lavage fluid associated with tissue injury and inflammation (Ward et al., 2001; Webb et al., 2001; Giannetti et al., 2004; Zhu et al., 2004; Boot et al., 2005). Though the function(s) of mammalian Ym proteins are not fully known, these novel lectin-binding proteins may play important roles in the pathogenesis of allergen-induced airway inflammation and remodeling (Zhu et al., 2004) and the regeneration of olfactory epithelium after toxicant-induced injury (Giannetti et al., 2004). More research is needed to fully understand the function and importance of these unique proteins in nasal epithelium after exposure to inhaled toxicants.

Cigarette smoke is another inhaled irritant that can alter the transitional epithelium in rodents. Unlike ozone, which predominantly induces mucous cell metaplasia, cigarette smoke exposure results in squamous metaplasia along with epithelial cell proliferation (Walker, 1983; Hotchkiss et al., 1995; Mauderly et al., 2004) (Figure 7). Chronic inhalation of formaldehyde also induces squamous metaplasia and epithelial cell proliferation in the nasal cavities of both rodents and nonhuman primates (Monticello et al., 1989, 1991).

It is important to remember that laboratory rodents exposed to highly water-soluble, gaseous irritants most often have lesions in the surface epithelium lining the lateral meatus (lateral margins of the naso- and maxilloturbinates and on the adjacent lateral wall) in the proximal aspects of the nasal cavity, which are covered by transitional epithelium. As mentioned previously, this regional sensitivity to injury may be due to (1) greater exposure of the tissue to the toxicant compared to other intranasal regions or (2) an inherently susceptible population of epithelial cells. With respect to ozone toxicity, Hotchkiss et al. have compared the regional differences in ozone-induced injury in nasal epithelia of the proximal nasal airways of the rat with predicted ozone dosimetry based on computational airflow-driven mass flux patterns that were developed by Kimbell et al. (Hotchkiss et al., 1994; Kimbell et al., 1993). The results of their study suggest that while dosimetry may play a role in the observed pattern of ozone-induced injury, much of the observed response must be due to inherent differences in epithelial susceptibility to ozone-induced injury. In contrast, cigarette smoke- and formaldehyde-induced nasal lesions are probably driven by intranasal airflow rather than by tissue susceptibility (Kimbell et al., 1997b).

Chemical-Induced Lesions of Respiratory Epithelium

As in transitional epithelium, lesions in respiratory epithelium may be superficial or extend to the underlying lamina propria. A common superficial, and often reversible, effect of irritants on respiratory epithelium involves attenuation and/or loss of cilia along the luminal surface in the proximal nasal cavity. This effect was frequently seen in mice and rats exposed to chlorine gas (Jiang et al., 1986) and was a common alteration observed in monkeys exposed to 0.15 and 0.30 ppm ozone for 6 or 90 days (8 hr/day) (Harkema et al., 1987c). Ciliated cell necrosis, mucous cell hyperplasia, and inflammatory cell influx (after 6 days of exposure only) were additional features in the nasal respiratory epithelium of monkeys exposed to ozone.

Studies have demonstrated that the nasal respiratory epithelium in both rodents and monkeys is susceptible to re-

peated exposures to formaldehyde, which result in loss of mucous cells and cilia, epithelial degeneration and necrosis, regenerative epithelial proliferation with or without squamous metaplasia, and an associated inflammatory response (Chang et al., 1983; Monticello et al., 1991). These nonneoplastic lesions in the proximal nasal cavity of rats, induced by acute or subchronic exposure, occurred in the same regions in which formaldehyde-induced nasal tumors were identified after chronic exposures to this irritant (Monticello and Morgan, 1997; Morgan et al., 1986a). In addition, formaldehyde-induced DNA-protein cross-links were also found in these same regions of respiratory and transitional mucosa, and not in the olfactory mucosa or bone marrow, of formaldehyde-exposed rats (Morgan, 1997). Though the role of DNA-protein cross-links in formaldehyde-induced nasal cancer is unclear, these cross-links may lead to DNA damage during cell replication (Heck et al., 1990).

In 1980, Swenberg and his colleagues at the Chemical Industry Institute of Toxicology (CIIT) in Research Triangle Park, North Carolina first published their findings that inhalation exposure of rats to formaldehyde induced nasal squamous cell carcinomas. As a consequence of this report, the Consumer Product Safety Commission issued a "Ban of Urea-Formaldehyde Foam Insulation (Commisson, 1982) and this led to long-term, multidisciplinary research efforts at CIIT and other institutions (e.g., TNO-CIVO Toxicology and Nutrition Institute in Zeist, Netherlands) to determine the underlying pathogenesis and cellular mechanisms of formaldehyde-induced tumors in rats, and the human health risk of formaldehyde. Subsequently, tremendous progress in the field of nasal toxicology has been made due to improved understanding of rat nasal anatomy, physiology, biochemistry, and pathology. In addition, this research led investigators to design innovative methods to better understand the influence of regional airway dosimetry on interspecies differences (e.g., rat vs. monkey) in toxic responses in the nose. The reader is referred to an excellent recent review, written by Morgan, on formaldehyde-induced carcinogenesis in relation to rat nasal pathology and human health risk assessment (Morgan, 1997).

Studies investigating formaldehyde-induced toxicity in rat nasal respiratory and transitional epithelium have shown that increases in cell proliferation occur after acute exposure and that alterations in cell proliferation are sensitive indicators for formaldehyde-induced toxic insults. Cytotoxic effects and increased epithelial cell proliferation appear to play an essential role in the development of formaldehyde-induced nasal carcinogenesis (Monticello et al., 1991; Monticello and Morgan, 1997). When using this animal data for human health assessment, it has been recommended that low concentration (<2 ppm airborne exposure) extrapolation, where no tissue damage was observed in animal studies, be uncoupled from the responses at high concentrations (>6 ppm), where epithelial degeneration, regenerative cell replication, and inflammation appear essential driving forces in formaldehyde-induced carcinogenesis (Morgan, 1997).

Chemical-Induced Alterations in Olfactory Epithelium

In the past two decades, there has been a considerable increase in the number of reports on the histopathology of nasal

olfactory mucosal responses to inhaled and parenterally administered chemicals (Bogdanffy, 1990; Gaskell, 1990; Mery et al., 1994a; Hastings, 2003). There has also been a concomitant increase in the understanding in the molecular and cellular mechanisms involved in olfaction (Buck and Axel, 1991; Mombaerts, 1999a; Buck, 2000, 2004) and the role of smell in such functions as memory and reproduction (Rekwot et al., 2001; Wilson and Stevenson, 2003; Wilson et al., 2004). In toxicology, interest has focused primarily on the role of olfactory mucosal metabolism in toxic processes in this target site. (Dahl and Hadley, 1991; Reed, 1993; Thornton-Manning and Dahl, 1997; Ding, 2003). However, interest in the toxicology of the olfactory mucosa has also been spurred by the observations that inhaled materials, including fine airborne particles, may enter the central nervous system via olfactory nerves (Evans and Hastings, 1992; Henriksson et al., 1997, 1999; Henriksson and Tjalve, 1998, 2000; Tjalve and Henriksson, 1999; Brenneman et al., 2000b; Oberdorster et al., 2002; Dorman et al., 2002).

Like the other nasal epithelia, non-neoplastic responses of the OE to toxic insult could include cellular degeneration, necrosis, atrophy, hyperplasia, and metaplasia (Gaskell, 1990). The type of alteration is dependent on the mechanism of action of the toxic agent and local dose. Nonspecific necrosis of all cells in the OE may occur after exposure to direct-acting irritants like chlorine (Jiang et al., 1983) and sulfur dioxide (Buckley et al., 1984; Giddens and Fairchild, 1972). In contrast, cell-specific toxicity in the olfactory epithelium may occur with degeneration and necrosis of either sensory or sustentacular cells. For example, the neurotoxin beta-beta'-iminodipropionitrile causes loss of sensory cells with sparing of sustentacular cells (Genter et al., 1992). In contrast, other toxicants, like dibasic esters and methyl bromide, may induce a primary effect on sustentacular cells with sensory-cell degeneration and necrosis occurring subsequent to the loss of support cells (Hurtt et al., 1988; Trela et al., 1992). The presence of cytochrome P-450-dependent monooxygenases in the OE also readily explains numerous observations of necrosis and loss of olfactory epithelium in laboratory animals after exposures to other indirect-acting agents like ferrocene, 3-methylfuran, acetaminophen, and nitrosodiethylamine (Dahl and Hadley, 1991).

For many inhaled chemical irritants, the OE lining the dorsal medial meatus is a common site of intranasal injury (Buckley et al., 1984; Hardisty et al., 1999). A histologic example of marked atrophy of the OE lining the dorsal medial meatus in a mouse that was repeatedly exposed to an inhaled toxicant is provided in Figure 8. Other OE toxicants may cause more complex intranasal patterns of OE injury whether or not the exposure is by way of inhalation or other routes of exposure (Hurtt et al., 1988; Mery et al., 1994b; Peele et al., 1991). In addition, the chemical-induced injury at the affected intranasal site may be restricted to the OE (Genter et al., 1992) and/or the underlying Bowman's Glands and the adjacent bone (Mery et al., 1994b).

Though most chemical insults to the OE induce necrosis (oncotic cell death) in OSNs and other OE cells, systemic administration of various cancer chemotherapeutic agents, such as vincristine sulfate and other tubulin-targeting anticancer drugs, have been recently reported to cause apoptosis of OSN with subsequent atrophy of the OE in mice (Kai et al.,

2004). Toxicant-induced apoptosis of OSN with subsequent OE atrophy has also been recently demonstrated in mice after a single, low dose (25 $\mu\text{g/kg}$ body weight) intranasal instillation of the trichothecene mycotoxin, satratoxin G, from the black mold *Stachybotrys chartarum* (Islam et al., 2006). Whether or not cell death of OSNs is induced by oncotic or apoptotic pathways, this will subsequently lead to OE atrophy and degeneration and atrophy of olfactory nerve fascicles in the underlying lamina propria and the olfactory nerve layer in the olfactory bulb. Of course, the magnitude of these secondary, peripheral neuronal lesions will be highly dependent on the severity and extent of the primary chemical-induced injury to the OSNs in the OE.

The severity of the OE damage and what cells are damaged will also determine if and to what extent the OE will regenerate after injury. In general, regeneration is characterized by an initial phase of proliferation of the residual basal cells, which may begin within 24 h of the chemical insult. Histologically proliferating basal cells will be larger and more basophilic than normal. With subsequent proliferation the regenerative epithelium will become thicker with randomly arranged cells. This will be followed by gradual differentiation of the newly regenerated cells with a gradual appearance of olfactory epithelial features. After a period of weeks to months the OE may recover to near normal morphology. The reader is referred to several more detailed descriptions of OE regeneration after injury caused by various chemical toxicants (Suzaki et al., 1997; Hurtt et al., 1988; Hext et al., 2001; Nan et al., 2001; Giannetti et al., 2004; Williams et al., 2004a, 2004b).

Certain modifications of the olfactory mucosa following epithelial necrosis and exfoliation may be adaptive in nature, leading to the development of a modified and possibly more resistant epithelial barrier to inhaled xenobiotic agents. These changes include squamous and respiratory metaplasia. Squamous metaplasia is a common response to injury in respiratory and transitional epithelium, but it occurs more rarely in the olfactory region. Respiratory metaplasia is a more common response in the olfactory region and leads to the development of an epithelial type that resembles respiratory epithelium (Figure 9).

Assessment of Human Risk from Inhaled Nasal Toxicants Using Animal Data

The estimation of human risk from inhaled toxicants is a complex process that may include direct observation of exposed people, but more often relies on extrapolations from toxicology studies using laboratory animal species such as rats and monkeys. McClellan (1995) has presented a research strategy for assessing human risk from the nasal toxicants that has been generally accepted and advocated by others. This strategy builds on a targeted approach to developing mechanistic data that will help elucidate quantitative linkages between exposure, dose, and response. When toxic responses in animals are site-specific, like most toxicant-induced nasal airway lesions, measured or predicted regional dose can be correlated with lesion location to evaluate the relative role of dose in the occurrence of the tissue responses. Assuming that there is a way to predict regional dose in humans, regional dose and knowledge of mechanism can then be used

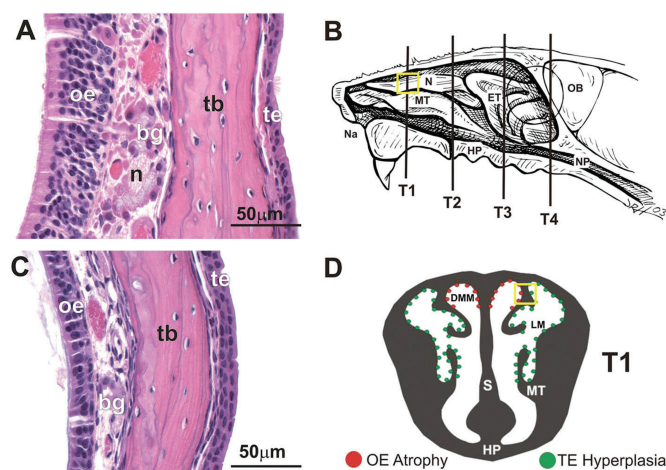


FIGURE 8.—Light photomicrographs of the dorsal aspect of nasoturbinate from mice repeatedly exposed to filtered air alone (A) or to an inhaled toxicant (C). The medial surface of each nasoturbinate is lined by olfactory epithelium (oe) and the lateral surface is lined by transitional epithelium (te). The toxicant-exposed nasoturbinate in C has marked atrophy of both the surface oe and its underlying lamina propria with noticeable loss or atrophy of Bowman's glands (bg) and nerve bundles (n) compared to the nasoturbinate in A that is lined by normal olfactory mucosa. In addition, (te) in C is mildly hyperplastic (2–3 cell thickness) compared to histologically normal te in A (1–2 cell thickness). The turbinate bone (tb) appears normal in both. The yellow square in (B) identifies the intranasal location of the microscopically examined site in the nasal tissue block (T1). (D) Diagrammatic representation of the anterior face of T1 identifying the distribution of the toxicant-induced lesions of (oe) and (te) and the site of the nasoturbinate examined in A and C.

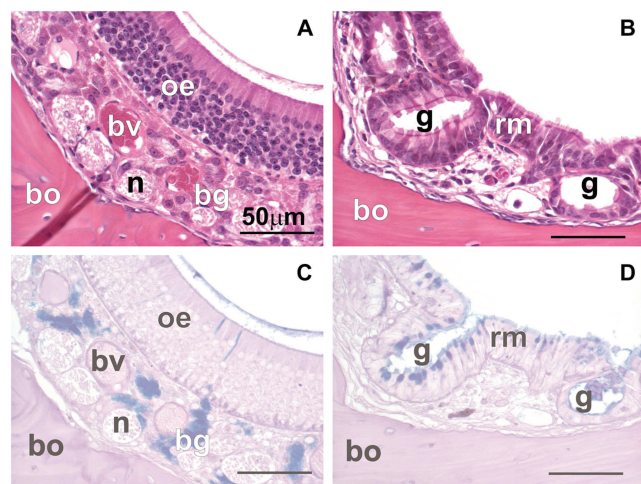


FIGURE 9.—Light photomicrographs of nasal epithelium lining the dorso-medial meatus in the proximal nasal airway (T1) of mice chronically exposed only to filtered air (A, C) or to an inhaled toxicant (B, D). Tissues in A and B were stained with hematoxylin and eosin, while tissues in C and D were stained with alcian blue (pH 2.4) and periodic acid Schiff (AB/PAS) to identify cells containing acidic and neutral mucosubstances. There is a marked loss of olfactory epithelium (oe) and Bowman's glands (bg) in the nasal mucosa from the mouse exposed to the inhaled toxicant (B). Oe is replaced by respiratory epithelium and subepithelial glands (g) indicating respiratory metaplasia (rm) of the oe. Notice that the AB/PAS-stained mucosubstances are normally restricted to the Bowman's glands (bg) and associated transepithelial ducts (C), but in rm the AB/PAS-stained mucosubstances are present in the mucous cells of the metaplastic glands and surface epithelium.

to accurately extrapolate animal data to people for human health risk assessment.

Critical to risk assessment of inhaled nasal toxicants is an accurate determination of dose to the targeted epithelial cells lining the nasal airways in the laboratory animal and an extrapolation from the animal dosimetric and cytotoxic data to risk for humans. Several groups of researchers have tackled the problem of nasal dosimetry by building on existing mathematical models or developing new computer-based approaches (Conolly et al., 2003, 2004; Frederick et al., 2001; Godo et al., 1995; Hotchkiss, 1994; Hubal et al., 1997; Kepler et al., 1998; Kimbell et al., 1993, 1997a, 1997b, 2001). Dose depends on mass transport of inhaled chemicals to the nasal airway lining and other factors including airflow, mucociliary clearance, and local metabolism. As mentioned previously in this chapter, the site-specific nature and primarily anterior-posterior gradient of nasal lesions induced by many inhaled toxicants, especially highly reactive, water-soluble toxicants (e.g., formaldehyde), suggests that intranasal airflow patterns play a major role in determining the location of toxicant-related lesions. To examine the effects of airflow on nasal toxicity, Kimbell and her colleagues have developed an anatomically accurate computational fluid dynamics (CFD) model of inspiratory airflow and regional gas uptake in the F344 rat nasal passages (Kimbell et al., 1997b) and more recently in the rhesus monkey (Kepler et al., 1998). Regional uptake patterns predicted by the CFD model matched the distribution of formaldehyde-induced lesions in the rat and monkey, providing evidence that airflow patterns played a major role in determining locations in these animal species.

Computer and experimental models of human nasal airways have been conducted by other investigators. Schreck et al. (1993) have conducted detailed measurement and analysis of flow fields and pressure drops in plastic replicas (3 times larger than normal) of human nasal airways. Other investigators have characterized and compared flow fields in a Styrofoam model (20 times the normal human nose) and a finite-element computer model (Hahn et al., 1993; Keyhani et al., 1995). When comparing the monkey nasal air flow patterns with those of humans, there were greater similarities than differences, as was predicted based the similarities in nasal gross anatomy between the 2 primate species.

By using these CFD models in animals along with nasal histopathology data, it is therefore possible to locate dose "hot-spots" that are associated with specific chemical-induced nasal lesions. Location of dose hot spots in human nasal airways for the same inhaled chemical can then be predicted, and dose levels of the predicted hot spots in humans can be compared to dose levels in animal airways that were determined experimentally to be toxic.

An understanding of the specific tissue/cell susceptibility and the underlying cellular/biochemical mechanisms responsible for the nasal toxicant-induced injury is also critical for accurate risk assessment. It is important to determine the similarities and differences in dose/response of morphologically similar nasal tissues/cells between the selected animal species and humans. Little information is currently available in this area and this must be one of the focus areas for nasal toxicology research in the future.

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