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# Biotransformation Enzymes in the Rodent Nasal Mucosa: The Value of a Histochemical Approach

by Matthew S. Bogdanffy*

**An increasing number of chemicals have been identified as being toxic to the nasal mucosa of rats. While many chemicals exert their effects only after inhalation exposure, others are toxic following systemic administration, suggesting that factors other than direct deposition on the nasal mucosa may be important in mechanisms of nasal toxicity. The mucosal lining of the nasal cavity consists of a heterogeneous population of ciliated and nonciliated cells, secretory cells, sensory cells, and glandular and other cell types. For chemicals that are metabolized in the nasal mucosa, the balance between metabolic activation and detoxication within a cell type may be a key factor in determining whether that cell type will be a target for toxicity. Recent research in the area of xenobiotic metabolism in nasal mucosa has demonstrated the presence of many enzymes previously described in other tissues. In particular, carboxylesterase, aldehyde dehydrogenase, cytochromes 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. This paper reviews the distribution of these enzymes within the nasal mucosa in the context of their contribution to xenobiotic metabolism. The localization of the enzymes by histochemical techniques has provided important information on the potential mechanism of action of esters, aldehydes, and cytochrome P-450 substrates known to injure the nasal mucosa.**

## Introduction

Research into mechanisms of chemical-induced nasal toxicity and carcinogenicity was promoted by the finding that agents of industrial and environmental importance such as formaldehyde, acetaldehyde, hexamethylphosphoramide, and 1,2-dibromoethane cause nasal tumors in rats following long-term inhalation exposure (*1–4*). Research interest in the mechanisms of nasal tumor formation revealed that nasal tumors could be produced by agents administered orally, including certain nitrosamines, 1,4-dioxane, and phenacetin (*5–7*). The latter studies were of particular importance since they suggested that agents reaching the nasal mucosa through the systemic circulation may be metabolically activated within target cells. Prior to 1982 when the group at Lovelace Inhalation Toxicology Research Institute first demonstrated the susceptibility of the rat and dog nasal mucosa to *N*-demethylate, a variety of cytochrome P-450 substrates, no other work had highlighted the potential importance of this tissue as a site for extrahepatic xenobiotic metabolism (*8,9*).

Since then, biotransformation rates in the nasal mucosa of several environmental pollutants and carcinogens have been quantitated. In general, these studies used homogenates prepared from nasal tissue excised from either the whole nasal cavity or from selected regions. While studies of this kind provide valuable information regarding the total activity of the mucosa, it is impossible to glean cell-specific information from such techniques. Since the nasal mucosa consists of heterogeneous cell populations, it is not clear whether any particular cell type is more metabolically competent than others. Also, it is not known if some cells may be at greater risk of chemical-induced toxicity than other more or less metabolically active cells. Culturing individual cells and measuring xenobiotic metabolism would be one approach to the question but currently this is not technically feasible as there are many (> 12) different cell populations in the nasal mucosa and methods are not available for their separation. However, histochemical analysis of the tissues, although not strictly quantitative, can provide cell-specific information. This review presents the toxicology and biochemistry of several examples of nasal toxicants and discusses how histochemical localization of biotransformation enzymes has aided in an understanding of the mechanism of action of these chemicals.

*E. I. du Point de Nemours and Co., Inc., P.O. Box 50, Elkton Road, Newark, DE 19714.

# Carboxylesterase

## Toxicology of Chronic Inhalation Exposure to Esters

Inhalation studies with glycol ether acetates, acrylate esters, dibasic esters, acetic acid, and acrylic acid have revealed a common response of the olfactory epithelium to both short-term and chronic exposure (*10–14*). In general, the olfactory epithelium undergoes a degenerative process with what appears to be initial loss of sensory cells. The olfactory epithelium lining the anterior portion of the dorsal meatus is frequently the first site injured, and the response occurs at airborne concentrations that do not damage the adjacent respiratory epithelium. Because conventional histological techniques have been employed in most of these studies, morphological details are inadequate to determine whether the sensory cells are truly the initial target site or whether the sustentacular cells (support cells) are first affected, followed by degeneration of the mature sensory cells. Evidence for the second scenario has come from recent studies in which electron microscopic evaluation of methyl bromide-induced sensory cell degeneration revealed that this lesion was secondary to a primary lesion in sustentacular cells (*15*). Ultrastructural studies are necessary to confirm this sequence of events in the case of the esters cited above. Hyperplastic responses have been noted in the basal cell layer following subchronic exposure to ethyl acrylate or dibasic esters, and these responses are often associated with respiratory metaplasia of the olfactory epithelium. This finding appears to be common to many agents damaging the olfactory epithelium and is most likely an adaptive response characteristic of epithelial regeneration. Regeneration of the olfactory epithelium to a morphologically normal architecture following cessation of exposure has been noted for several chemicals such as methyl bromide (*16*), methyl isocyanate (*17,18*), and the dibasic esters (*12*).

## Metabolism of Inhaled Esters

Several examples support the hypothesis that carboxylesterases, located within the nasal mucosa, hydrolyze inhaled esters to their corresponding acids, which may contribute to the histopathological changes seen following inhalation exposure. Propylene glycol monomethyl ether acetate (PGMEA) is readily absorbed in the upper respiratory tract upon inhalation (*19*) and is hydrolyzed to propylene glycol monomethyl ether and acetic acid (*10,13*). Inhalation exposure of rats and mice to the parent compound, PGMEA, induces lesions of the olfactory epithelium. Similar inhalation studies with the hydrolysis products showed that propylene glycol monomethyl ether has no effects on nasal olfactory epithelium, while acetic acid induces the same olfactory epithelial lesion as PGMEA (*13*).

Both ethyl acrylate and its hydrolysis product acrylic acid induce lesions in the nasal mucosa that are restricted to the olfactory epithelium (*11*). No reports could be identified from the literature to suggest that inhalation exposure to the alcohol metabolite of ethyl acrylate, ethanol, is toxic to the nasal epithelium. The absorption of ethyl acrylate by the upper respiratory tract of rats is saturable and is reduced by pretreatment with the carboxylesterase inhibitor, triorthocresyl phosphate (*19*). Furthermore, hydrolysis of ethyl acrylate to ethanol and acrylic acid by nasal carboxylesterase *in vitro* was demonstrated to be significant (*13*).

The dibasic esters (DBE) are a mixture of dimethyl adipate, dimethyl glutarate, and dimethyl succinate. Ninety-day inhalation exposure of rats to DBE resulted in lesions of the olfactory epithelium at concentrations that do not affect the respiratory epithelium (*12*). The lesions are primarily of the olfactory sensory cell layer and are similar in morphological characteristics to those produced by PGMEA and ethyl acrylate. Metabolism of DBEs by nasal respiratory and olfactory mucosae *in vitro* demonstrated that hydrolysis of DBEs yields mainly the monomethyl esters (monomethyl adipate, monomethyl glutarate, and monomethyl succinate) (*20*). Hydrolysis of the remaining ester linkage to form the diacids does not appear to occur. As shown in Table 1, the carboxylesterase activity of olfactory mucosa is generally much greater than respiratory mucosa, suggesting that local formation of acid metabolites of the DBEs in olfactory mucosa may be the initial biochemical event preceding sensory cell degeneration (*21–23*). The difference in carboxylesterase capacity between respiratory and olfactory mucosa may thus account, in part, for the increased susceptibility of olfactory tissue to the toxic effects of inhaled esters.

## Histochemistry of Carboxylesterase

The biochemical studies discussed above were performed with homogenates prepared from nasal tissue. The question remains from these studies as to which cell type(s) within the mucosa contain carboxylesterase activity. Recently, cold glycol methacrylate embedding procedures have enabled us to obtain improved morphological detail of nasal epithelium while preserving

**Table 1. Hydrolysis of several esters by nasal mucosal carboxylesterase.**

| Substrate | Activity[a] | |
|---|---|---|
| | Respiratory | Olfactory |
| Dimethyl succinate[b] | 0.362 | 3.222 |
| Dimethyl glutarate[b] | 0.683 | 4.269 |
| Dimethyl adipate[b] | 0.382 | 2.014 |
| *p*-Nitrophenyl butyrate[b,c] | 0.099 | 0.605 |
| Amyl acetate[d,e] | 0.100 | 0.120 |
| Phenyl acetate[d,e] | 0.230 | 0.250 |
| β-Butyrolactone[d,e] | 0.063 | 0.064 |

[a]Values are expressed as μmole/min/mg protein.
[b]Activity in whole homogenate.
[c]From Bogdanffy et al. (*21*).
[d]Activity in S-9 fraction.
[e]From Dahl et al. (*22*).

enzymes for histochemical localization (*23*). This technique was applied in a detailed study of the distribution of nonspecific esterase (α-naphthyl butyrate esterase, NBE) in the nasal mucosa (*21*). Differences in localization and intensity of carboxylesterase reaction product across the variety of epithelial cell types were observed.

The α-naphthyl butyrate esterase reaction product was found to be variable in epithelial cells of the respiratory mucosa (Plate 1, Table 2). Ciliated and nonciliated columnar respiratory epithelial cells and basal cells stained positively, with the stain being more intense in the ciliated cells. The reaction product was distributed evenly throughout the cytoplasm but was noticeably weaker in the nuclei. Goblet cells were the one respiratory epithelial cell type devoid of the cytoplasmic reaction product. The apparent lack of activity in goblet cells may be a result of the decreased cytoplasmic volume of the cells. In most cell types the reaction product appeared mainly in cytoplasm, but it was only evident along the cell wall in goblet cells. Interestingly, mucoid material overlying the portion of airway lined by respiratory mucosa stained positively for NBE, as did seromucous glands of the underlying mucosa. The enzyme present in the mucus, therefore, is probably secreted by the acinar cells of the seromucous glands.

In olfactory epithelium, two distinct layers of NBE activity were apparent (Plate 2). The luminal aspect of the epithelium, predominantly in the apical cytoplasm of sustentacular cells, was moderately positive for NBE. Sustentacular cells have footlike projections extending down to the basal cell layer that also demonstrated NBE activity. In contrast to respiratory epithelium, the basal cells of olfactory epithelium were negative for NBE. In the lamina propria, Bowman's glands stained intensely. The reaction product was concentrated in the acinar cells and the cells lining the ducts; however, the lumen of these ducts containing secretions of Bowman's glands did not show any NBE activity.

Histochemical localization of carboxylesterase activity showed that specific cell types in both respiratory and olfactory mucosa have the capacity of hydrolyze esters while other cell types are apparently devoid of activity. Within olfactory mucosa, the primary site of ester-induced lesions, sustentacular cells and acinar cells of Bowman's glands may be the initial site of toxic acid formation. Esterase activity in sustentacular cells and ducts of Bowman's glands is particularly important in that these cells are so closely associated with the sensory cells. It is interesting that sensory cells themselves do not appear to possess esterase activity. Possibly the esterase activity exists in the olfactory vesicles of sensory cells; however, these structures cannot be clearly distinguished at the light microscopic level. Ultrastructural evaluation of the enzyme distribution is needed to answer this question. If olfactory vesicles do not possess esterase activity then, in some manner, locally generated acids diffuse or are transported into neuronal cells. Sustentacular cells may communicate with sensory cells through tight junctions (*24*). Perhaps through this mechanism, sustentacular cells bring esterase activity, and, therefore, acid metabolites into close proximity to the sensory cells where such metabolites may be transported via tight junctions directly into the sensory cells. Through this type of transport, acid metabolites released from PGMEA, EA, or the DBEs in the neighboring cells may cause cytotoxicity to the sensory cells. The mechanism for such transport and the critical subcellular target of these acids have not been elucidated but deserve further attention.

## Aldehyde Dehydrogenase

### Toxicology of Inhaled Aldehydes

Two simple aldehydes, acetaldehyde and formaldehyde, have been shown to induce carcinomas of the nasal cavity upon long-term inhalation exposure (*1,2*). In the case of acetaldehyde, the tumors are both squamous cell carcinomas and adenocarcinomas, arising from the posterior portion of the nasal cavity (*2*). In the case of formaldehyde, the tumors are squamous cell carcinomas originating from the anterior portion of the nasal mucosa (*1*). Differences in nonneoplastic responses to these two agents are evident at low toxic concentrations, which for formaldehyde is 2 to 3 ppm and for acetaldehyde is 400 to 1000 ppm.

**Table 2. Histochemical distribution of biotransformation enzymes in the rat nose.[a,b]**

| Cell type | AldH | FdH | NBE | P-450 red | P-450 | EH | GSHt | BαPOH |
|---|---|---|---|---|---|---|---|---|
| *Squamous epithelium* | + | ND | + | ND | ND | ND | ND | ND |
| Respiratory epithelium | +++ | ✓[b] | ++ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Seromucous glands | ND | ND | + | ✓ | ✓ | ✓ | ✓ | ✓ |
| Olfactory sustentacular cells | – | ✓ | ++ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Olfactory nerves | – | ✓ | – | – | – | – | – | – |
| Bowman's glands | + | ✓ | +++ | ✓ | ✓ | ✓ | ✓ | ✓ |

[a]Abbreviations and references are: AldH, acetaldehyde dehydrogenase (*31*); FdH, formaldehyde dehydrogenaase (*35*); NBE, α-naphthyl butyrate esterase (*21*); P-450 red, NADPH-cytochrome P-450 reductase (*52*); P-450, several inducible forms of cytochrome P-450 (*52–54*); EH, epoxide hydrolase (*52*); GSHt, several inducible forms of glutathione *S*-transferases (*52*), BαPOH, benzo(*a*)pyrene hydroxylase (*52*).

[b]For studies in which qualitative evaluations were made of stain intensity, the scores are: –, no reaction; +, weak action; ++, moderately strong reaction; +++, strong reaction. For studies in which no qualitative evaluation was made, ✓ and – indicate enzyme present or absent, respectively.

Earlier signs of toxicity highlight differing susceptibilities of the respiratory versus olfactory tissue to the effects of acetaldehyde and formaldehyde. Acetaldehyde exposure for approximately 4 weeks at low concentrations (400 ppm) induces lesions primarily of olfactory epithelium, characterized by a loss of microvilli; epithelial disarrangement; and the appearance of irregularly shaped nuclei, loss of sensory cells and, at higher concentrations (5000 ppm), focal hyperplasia and squamous metaplasia (*25*).

Short-term (4-week) formaldehyde exposure, on the other hand, induces cytotoxicity in the respiratory epithelium (*26*). After 3 days of exposure to 6, 10, 15 or 20 ppm formaldehyde, an increase in cell replication rate is evident in the respiratory epithelium lining the anterior portion of the naso- and maxilloturbinates (*26,27*). The effects progress, following 13 weeks of 10 and 20 ppm formaldehyde to hyperplasia and squamous metaplasia, respectively (*28*). Significant changes in olfactory epithelium do not occur until 13 weeks of exposure to 20 ppm (*28*).

## Metabolism of Acetaldehyde and Formaldehyde

Regional differences in cytotoxicity and tumor formation induced by formaldehyde and acetaldehyde have, for many years, been attributed, at least in part, to differing water solubilities of the two compounds and hence differing patterns of deposition of the two within the nasal cavity. However, this cannot entirely explain the regional differences in response. In the case of acetaldehyde, studies with radiolabeled material have shown deposition throughout the nasal mucosa (*29*). Although the influence of water solubility on deposition patterns within the nose is likely a major factor contributing to the difference in lesion distribution between the two compounds, regional differences in the nasal metabolism of formaldehyde and acetaldehyde may also offer partial explanation for the effects.

Both acetaldehyde and formaldehyde are highly reactive compounds, so it follows that removal of absorbed acetaldehyde or formaldehyde through metabolic processes will protect the epithelium. The oxidative detoxication of acetaldehyde and formaldehyde by rat nasal respiratory and olfactory mucosal homogenates has been investigated (*30*). Two isozymes of acetaldehyde dehydrogenase were detected in rat nasal respiratory and olfactory mucosae. The high $K_m$ isozyme is probably most responsible for detoxication of inhaled acetaldehyde, while the low $K_m$ isozyme may be the same enzyme that catalyzes the oxidation of formaldehyde (*30*). There is approximately a 5-fold difference in the activity of acetaldehyde dehydrogenase between respiratory and olfactory mucosae (Table 3). This difference suggests that regions of the nasal cavity covered by respiratory mucosa are better equipped to detoxify inhaled acetaldehyde than those lined by olfactory mucosa. The pathology of acute and chronic exposure to acetaldehyde supports this biochemical finding.

The formaldehyde dehydrogenase-mediated oxidation of formaldehyde is glutathione dependent. The true substrate for the reaction is *S*-hydroxymethyl glutathione, formed by the condensation of formaldehyde and glutathione. The oxidation of formaldehyde by rat nasal olfactory mucosa is approximately 2-fold higher than that for respiratory mucosa when glutathione is included in the reaction mixture (Table 2). This result suggests the olfactory mucosa is better equipped than the respiratory mucosa to detoxify inhaled formaldehyde under conditions of regular glutathione status, i.e., not depleted [9 days of exposure to 6 ppm formaldehyde does not reduce nasal respiratory mucosal glutathione levels (*30*)]. The pathologic findings in the respiratory mucosa discussed above are consistent with this conclusion.

## Histochemistry of Aldehyde Dehydrogenase

The low acetaldehyde dehydrogenase activity in the nasal olfactory mucosa and the high activity in respiratory mucosa was demonstrated biochemically and was subsequently confirmed histochemically by means of $NAD^+$-dependent reduction of nitro-blue tetrazolium, using cold glycol methacrylate embedded sections of rat nose (*31*). In olfactory mucosa, little if any reaction product was detected in cells of the epithelium or lamina propria (Plate 3, Table 2). Only a weak deposition of formazan granules was detected in acinar cells of Bowman's glands, the cells lining the intraepithelial portion of the ducts of Bowman's glands, basal cells, and occasional globose sensory cells. Sustentacular cells and mature sensory neurons were negative for aldehyde dehydrogenase, suggesting a paucity of acetaldehyde detoxication capacity in the olfactory mucosa.

The most interesting aldehyde dehydrogenase staining patterns were observed in the respiratory mucosa (Plate 3). Only minimal activity was located in the subepithelial seromucous glands, while most of the activity was confined to ciliated cells of the epithelial layer. The formazan reaction product was polarized toward the luminal aspect of the cells but did not appear to be located in the ciliary basal bodies. In goblet cells, the reaction product was confined to the small amount of cytoplasm surrounding the secretory vacuole. Aldehyde dehydrogenase activity was also detected in nonciliated cells, although the pattern of staining was more diffuse throughout the cell cytoplasm.

Ciliated respiratory epithelial cells are known to contain a large amount of mitochondria that provides the energy required to drive ciliary movement (*32*). The location of these mitochondria reflects the formazan granule deposition pattern formed by the aldehyde dehydrogenase staining reaction. For this reason and the fact that mitochondria are known to possess several aldehyde dehydrogenase isozymes (*33*), it is believed that the histochemical stain is localizing primarily mitochondrial aldehyde dehydrogenase. Cytosolic forms of aldehyde dehydrogenase and forms bound to smooth endoplasmic reticulum have also been identified in small quantities

Table 3. Kinetic constants for oxidation of *S*-hydroxymethyl glutathione, formaldehyde, and acetaldehyde by rat nasal respiratory and olfactory mucosae.[a]

| Enzyme | Substrate | Tissue | $V_{max}$ | $K_m$ |
|---|---|---|---|---|
| FdH | *S*-Hydroxymethyl glutathione | Respiratory | 0.90 | 0.0026 |
| | | Olfactory | 1.77 | 0.0026 |
| AldH | Formaldehyde | Respiratory | 4.07 | 0.481 |
| | | Olfactory | 4.39 | 0.647 |
| AcldH I | Acetaldehyde | Respiratory | 128 | 20 |
| | | Olfactory | 28 | 22 |
| AcldH II | Acetaldehyde | Respiratory | 0.8 | 0.0003 |
| | | Olfactory | 2.2 | 0.1 |

[a]From Casanova-Schmitz et al. (*30*).

(*34*), and these may contribute to the more diffuse staining noted in the nonciliated cells. It is interesting to note, however, that no reaction product was detected in the luminal aspect of sensory cells that are also ciliated. These histochemical studies point out the differences between cells in terms of their ability to detoxify acetaldehyde. In this respect, the ciliated respiratory epithelial cells are likely to be the main cell type contributing to the respiratory mucosa's ability to detoxify inhaled acetaldehyde.

A recent report described the distribution of formaldehyde dehydrogenase activity in nasal mucosa in which tissues were stained in the presence and absence of glutathione (Table 2) (*35*). In contrast to acetaldehyde dehydrogenase, formaldehyde dehydrogenase was detected as a diffuse cytoplasmic stain in both respiratory and olfactory epithelial cells, Bowman's glands, and seromucous glands when glutathione was included in the reaction mixture. The omission of glutathione blocked the formation of formazan, demonstrating that the reaction product represents specifically formaldehyde dehydrogenase.

The biochemical findings taken together with the histochemical studies of acetaldehyde and formaldehyde dehydrogenase show that the respiratory and olfactory mucosae differ significantly in the relative activity and distribution of these enzymes. Although the substrates for these enzymes are structurally similar, their metabolism and the cells responsible for their metabolism vary considerably between the two mucosal types. However, for both acetaldehyde dehydrogenase and formaldehyde dehydrogenase, the distribution of these detoxication enzymes correlates with regional resistance to the toxic effects of inhaled acetaldehyde and formaldehyde. These studies provide valuable clues as to the mechanism of action of these compounds by quantitating detoxication capacity within a tissue and identifying specific cell types within the tissue responsible for the enzyme activity.

# Cytochrome P-450 and Related Enzymes

## Toxicologic Responses to Cytochrome P-450 Substrates

The response of the nasal cavity to reactive metabolites that are formed as a result of cytochrome P-450 activation is not as clear as that for the esters and aldehydes noted above; this is, in part, due to the diversity of compounds known to be activated by this group of enzymes which, in turn, is a result of the broad spectrum of cytochrome P-450 isozymes. Localization of cytochrome P-450 isozymes within specific cell types may define cell populations within the mucosa as targets for particular chemicals. Pathologic responses to nasal carcinogens such as nitrosamines and haloalkenes, which are known to be metabolically activated to reactive intermediates by cytochrome P-450, have been reviewed (*36,37*). As mentioned below, several isozymes of cytochrome P-450 have been identified in nasal mucosa.

## Metabolism of P-450 Substrates

By far, the most extensively studied nasal cytochrome P-450 substrates are the nitrosamines and polycyclic aromatic hydrocarbons—both of which are environmental contaminants and nasal carcinogens. For example, inhalation exposure of hamsters to benzo(*a*)pyrene produces a high incidence of nasal cavity and alimentary tract tumors (*38*). The metabolism of benzo(*a*)pyrene has been studied in the respiratory and olfactory tissue of the hamster and rat (*39,40*). Conversion of benzo(*a*)pyrene to mutagenic metabolites was demonstrated in regions of the nasal cavity lined by both respiratory and olfactory mucosa. Mutagenic metabolites also appear in nasal mucus where they may be transported to the alimentary tract exposing esophageal and gastric epithelium to ultimate carcinogenic metabolites of benzo(*a*)pyrene.

Species differences in the oxidation of cytochrome P-450 substrates by nasal tissue have been observed (*41*), and these are particularly evident when examining the metabolism of benzo(*a*)pyrene. The total metabolism of benzo(*a*)pyrene is approximately equivalent for maxilloturbinate (respiratory mucosa) or ethmoturbinate (olfactory mucosa) tissue in the Syrian hamster (*40*). In the dog, however, there is a 7-fold increase in total benzo(*a*)pyrene metabolism in ethmoturbinate tissue relative to maxilloturbinate tissue (*42*). Similarly, species differences exist in the *N*-deethylation of diethylnitrosamine by nasal mucosa and the difference correlates with nasal tumor susceptibility. Syrian hamster nasal mucosa is more efficient than that of the Sprague-Dawley rat for diethylnitrosamine deethylation,

and the former species is more susceptible to diethylnitrosamine-induced tumors than is the latter (*43*).

The tobacco-specific nitrosamine 4-(*N*-methyl-*N*-nitrosamino)-1-(3-pyridyl)-1-butanone (NNK) (*44*), a known nasal carcinogen in rats, undergoes extensive cytochrome P-450-dependent *N*-dealkylation reactions in both respiratory and olfactory mucosa, liberating DNA-reactive alkylating agents. Differences in the efficiency of DNA alkylation by NNK between respiratory mucosa is two to four times greater than in olfactory mucosa, following a single dose of NNK (*45*). Intraperitoneal administration of NNK results in a sequestration of radioactivity in the acinar cells and ducts of Bowman's glands, sustentacular cells of olfactory mucosa, and seromucous glands of the respiratory mucosa (*46*).

Histoautoradiographic studies of the distribution of radiolabeled nasal carcinogens yielded the first clues that cytochrome P-450-mediated biotransformation of chemicals varies from one cell type to another. Nitrosodiethanolamine follows a pattern of distribution similar to that of NNK (*47*). The oral analgesic drug and rat nasal carcinogen phenacetin also concentrates primarily in Bowman's glands, but *in vitro* studies indicate binding to sustentacular cells and goblet cells of the respiratory epithelium as well (*48*). A recent study demonstrated the toxic effects to olfactory mucosa of the phenacetin-precursor acetaminophen (*49*). Together these reports are suggestive of a high concentration of cytochrome P-450 in Bowman's glands and lower, although significant, levels in sustentacular cells, seromucous glands, and selected cells of the respiratory epithelium.

Biochemical measurement of cytochrome P-450 content in nasal mucosa has confirmed the high concentration inferred by the metabolism studies. Cytochrome P-450 concentration in rat nasal olfactory mucosa is approximately 240 pmole/mg protein and is about seven times higher than respiratory mucosa (*50*). Total cytochrome P-450 content of rabbit olfactory mucosa has been reported to be as high as 750 pmole/mg protein (*51*). In many species, nasal P-450 concentration in the respiratory and olfactory mucosae combined is second only to that of the liver (*50*). As with other biochemical measurements, these studies do not provide information on the cellular specificity of cytochrome P-450.

## Immunohistochemical Localization of Cytochrome P-450 and Related Enzymes

Advances in immunohistochemical staining techniques have enabled the identification of cytochrome P-450 isozymes in rat nasal mucosa (Table 2). In all studies the antibody has been raised against purified rat hepatic cytochrome P-450 and, therefore, recognizes antigens in nasal mucosa of immunochemical similarity to those in liver. The phenobarbital-inducible isozyme of cytochrome P-450 and NADPH-cytochrome P-450 reductase and the β-naphthoflavone-inducible form of P-450 have been studied (*52–54*). The pattern of enzyme distribution for both P-450 isozymes is similar to that for NBE and parallels the distribution of covalently bound radiolabeled compounds known to be metabolized to reactive products by cytochrome P-450. In respiratory mucosa, most cell types in the epithelium and cells of the seromucous glands stain positively for cytochrome P-450, although less intensely than olfactory mucosa. In olfactory mucosa, the sustentacular cells and acinar cells of Bowman's glands and ducts are also positive for cytochrome P-450. Similar to NBE, basal cells of the olfactory epithelium stain negatively for P-450. The distribution of benzo(a)pyrene hydroxylase (BαPOH), a cytochrome P-450-dependent monooxygenase catalyzing the hydroxylation of the polycyclic aromatic carcinogen benzo(*a*)pyrene, corresponds with that of P-450 (*53*).

Two other enzymes related to metabolic activation and detoxication of chemicals, epoxide hydrolase (EH) and glutathione *S*-transferase (GSHt), have also been localized immunocytochemically (*53*). Epoxide hydrolase catalyzes the hydration of electrophilic epoxide moieties to vicinal diols in alkene and arene molecules. Hydrolysis of the epoxide by EH is, in most cases, a detoxication reaction, but in some cases may lead to substrates for further metabolic activation, such as the diol epoxide metabolite of benzo(*a*)pyrene. Similarly, GSHt, which catalyzes the conjugation of glutathione with electrophilic centers facilitating water solubility and excretion may also form more toxic end products. Both EH and GSHt have patterns of distribution similar to the cytochrome P-450 enzymes. Therefore, in the case of EH- or GSHt-mediated detoxication of chemicals activated by P-450, the nasal mucosa, with the exception of sensory cells, is afforded some degree of protection. In the case where EH and GSHt result in more biologically active metabolites, the nasal mucosa may be a target site for chemical-induced toxicity.

## Conclusion

An increasing number of chemicals that are toxic to the rodent nasal mucosa are being identified through routine testing. The relevance of this toxicity to human health risk is not clear but will become more apparent through mechanistic research, including studies of nasal xenobiotic biotransformation. A wide variety of biotransformation enzymes such as aldehyde dehydrogenases, esterases, monooxygenases, epoxide hydrolases, and conjugation enzymes have recently been identified in the nasal mucosa by enzyme histochemical and immunohistochemical techniques. Localization of these enzymes is providing information on specific cellular targets. Furthermore, the high capacity for xenobiotic metabolism in nasal mucosa, compared with other tissues such as the liver, suggests that nasal metabolism is a significant factor. Ultimately, studies with human and nonhuman primate tissue will be necessary to complete the extrapolation of metabolic factors from rodents to humans.

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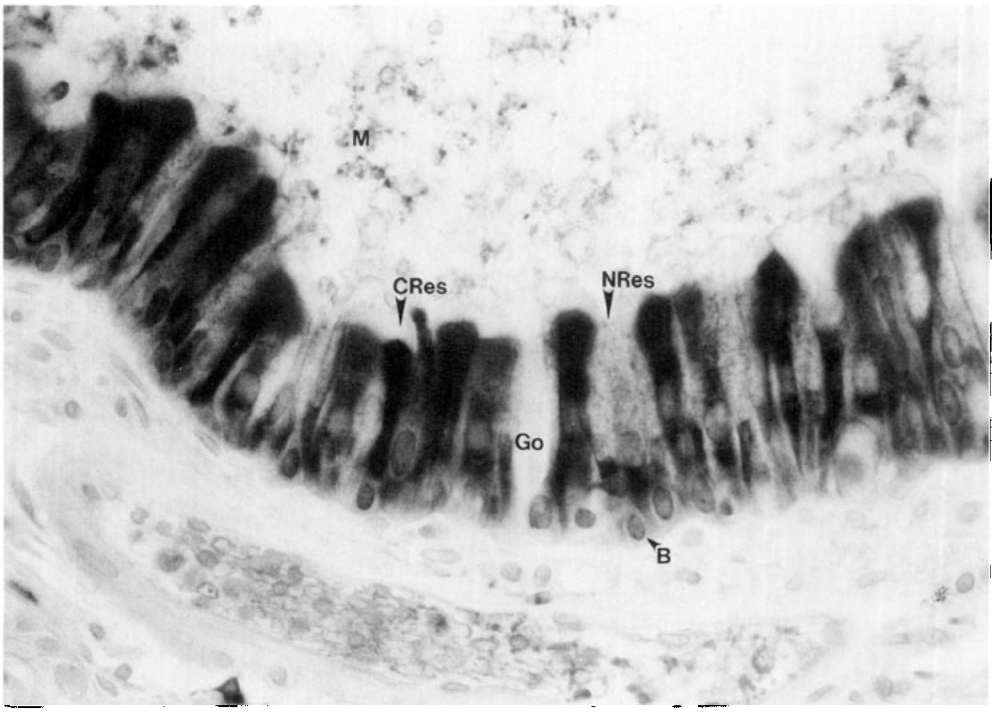


PLATE 1. α-Naphthyl butyrate esterase histochemical stain of male rat respiratory epithelium. Moderate product formation and even distribution of reaction product is evident in ciliated respiratory epithelial cells (CRes). Carboxylesterase was weak but evident in nonciliated respiratory epithelial cells (NRes). Basal cells (B) showed weak activity while goblet cells (Go) were weak to negative. Mucus secretions (M) were weakly positive for carboxylesterase, as were the seromucous glands in the underlying mucosa (not shown). ×570. From Bogdanffy et al. (*21*).

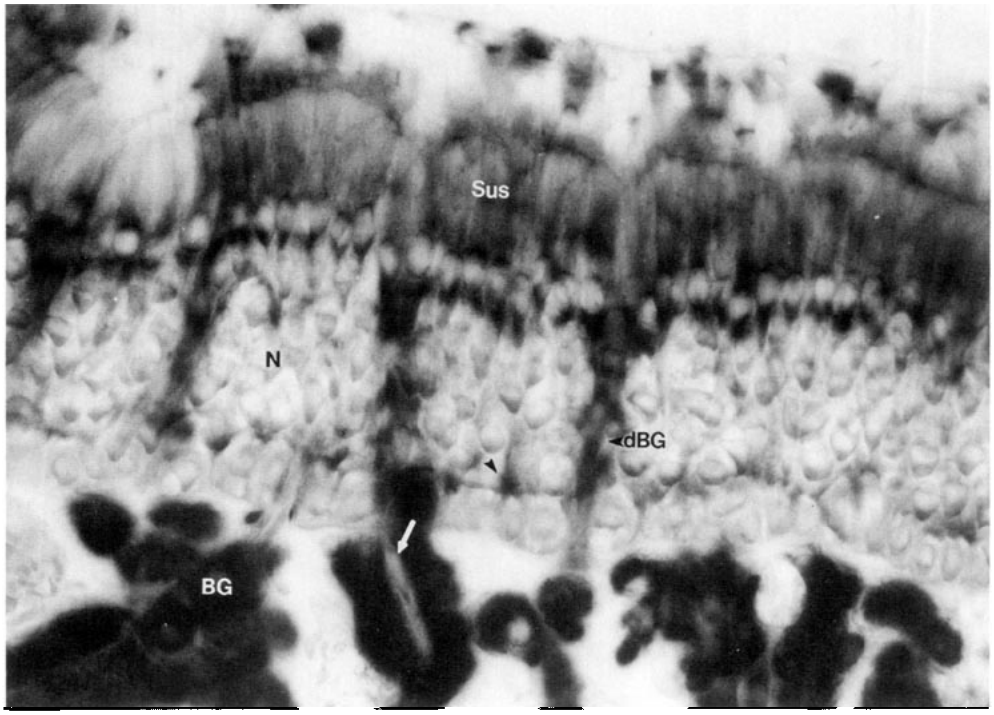


PLATE 2. α-Naphthyl butyrate esterase histochemical stain in mail rat olfactory mucosa. Sustentacular cells (Sus) and their footlike projections above the basal cell layer (arrowhead) were moderately positive. The lumen of the ducts (arrow) were negative, as was the neuronal cell layer (N). ×570. From Bogdanffy et al. (*21*).

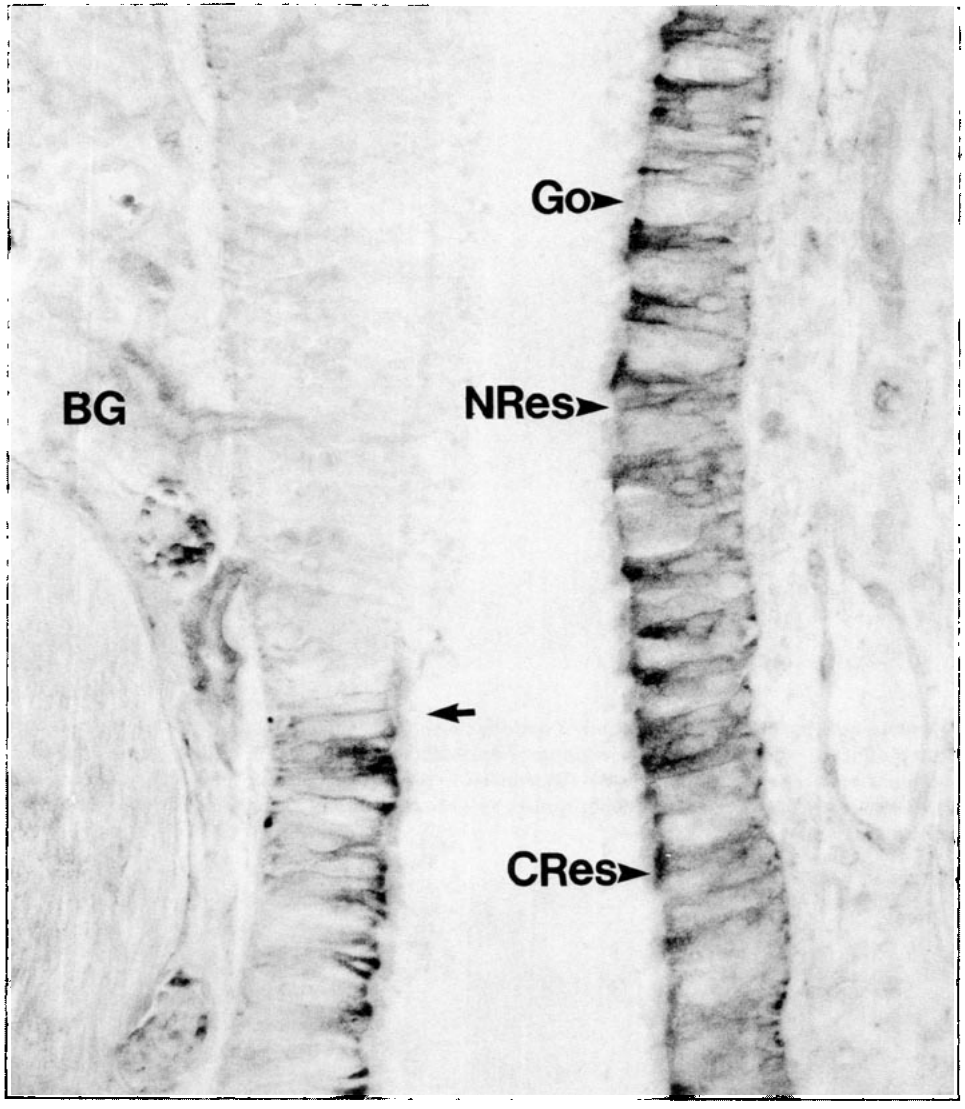


PLATE 3. Acetaldehyde dehydrogenase histochemical stain in male rat respiratory and olfactory mucosae. The reaction product is present in luminal aspects of ciliated respiratory epithelial cells (CRes). A moderate amount of activity can be seen in the nonciliated respiratory epithelial cells (NRes). Enzyme activity is weak in the cytoplasm surrounding the goblet cells (Go), and it is weak but apparent in the underlying Bowman's glands (BG). The respiratory-olfactory epithelial junction is apparent (arrow) along the epithelium lining the septum. ×450. From Bogdanffy et at. (*31*).