XENOBIOTIC METABOLISM IN THE NASAL EPITHELIA

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SUMMARY

The nasal epithelia in several species contains fairly high levels of drug metabolising enzymes, and in many cases the site specific toxicities of chemicals are due to their metabolic activation in the nasal tissues. This article reviews some of the current literature on the metabolic capacities of nasal epithelia, in particular the distribution and characteristics of cytochrome P-450 isozymes. In addition to the role of these nasal enzymes in metabolism of inhaled xenobiotics, other possible biological roles for these enzymes are also discussed.

I. INTRODUCTION

Together with the skin and the gut the respiratory tract forms the barrier between the organism and its environment. These barrier tissues receive a wide variety of chemical insult as a result of their biological role. Many chemicals are present in the inspired air as vapours and/or aerosols which gain ready access to the cells of the respiratory tract. It is not surprising therefore that the lungs and linings of the respiratory tract are capable of metabolising foreign compounds. Research over the last ten years into the metabolic activities in the linings of the upper respiratory tract has shown high activities of xenobiotic metabolising enzymes, notably the cytochrome P-450 isozymes, in nasal epithelia and in particular in the olfactory epithelium.

The first evidence that the nasal epithelia might be capable of metabolising xenobiotics became available in the late 1960's. Studies of the preferential generation of tumours in the olfactory mucosa and lining of the respiratory tract by N,D-diethylnitrosamine /1/, combined with the knowledge that nitrosamines required metabolic activation to induce tumours, led to the suggestion that activated products were being produced in the liver and carried in the blood to the respiratory tract. Later studies of the distribution of radiolabelled nitrosamines, e.g. N-nitrosonornicotine /2/, showed conclusively the selective accumulation of tissue bound metabolic products in nasal epithelia. These metabolic products, namely N-hydroxynitrosamines, were known to result from cytochrome P-450

mediated oxidation, and this enzyme was subsequently identified in nasal microsomes /3/.

The following article reviews some of the literature available on the metabolic capacities of nasal epithelia and draws some conclusions as to the biological role of these activities. The discussion is prefaced with a short description of the anatomy of the nasal passages.

IL ANATOMY OF THE NASAL PASSAGES

The nasal passages have a complex anatomy of cartilaginous convolutions and sinuses that fulfil the functions of filtering and warming inspired air, as well as allowing it access to the olfactory epithelium. In man the precise arrangement of the cartilage and shapes of the sinuses is different between different races and is probably influenced to a degree by the climate in which each race has evolved.

In man the nasal passages open into the external environment through the nostrils (Figure 1). The nostrils extend caudally to the nasal valve (ostium internum). In the adult human the passages continue caudally, divided by the nasal septum, between 10 and 14 cm to the end of the nasal septum and the beginning of the nasopharynx. The nasopharynx begins posterior to the nasal septum and extends caudally to the start of the trachea and oesophagus. Between the nasal valve and the nasopharynx the nasal passages fill the vertical space between the hard palate and the cribriform plate, which lies immediately below the brain with convolutions of epithelium covered bone and cartilage.

Two types of epithelia line the nasal passages. Olfactory epithelium line the areas responsible for the detection of odours whilst respiratory epithelium lines the remainder and is continuous with the rest of the respiratory tract. The cellular anatomy of the olfactory epithelium is relatively simple. It is a pseudo stratified epithelium consisting of a layer of basal cells which are mitotically active below layers of neurones and supporting Sustentacular cells (Figure 2). Sustentacular cells have microvilli at the external surface and a layer of mucus covers the epithelium which is produced by the mucus secreting Bowmans glands which lie below the epithelium.

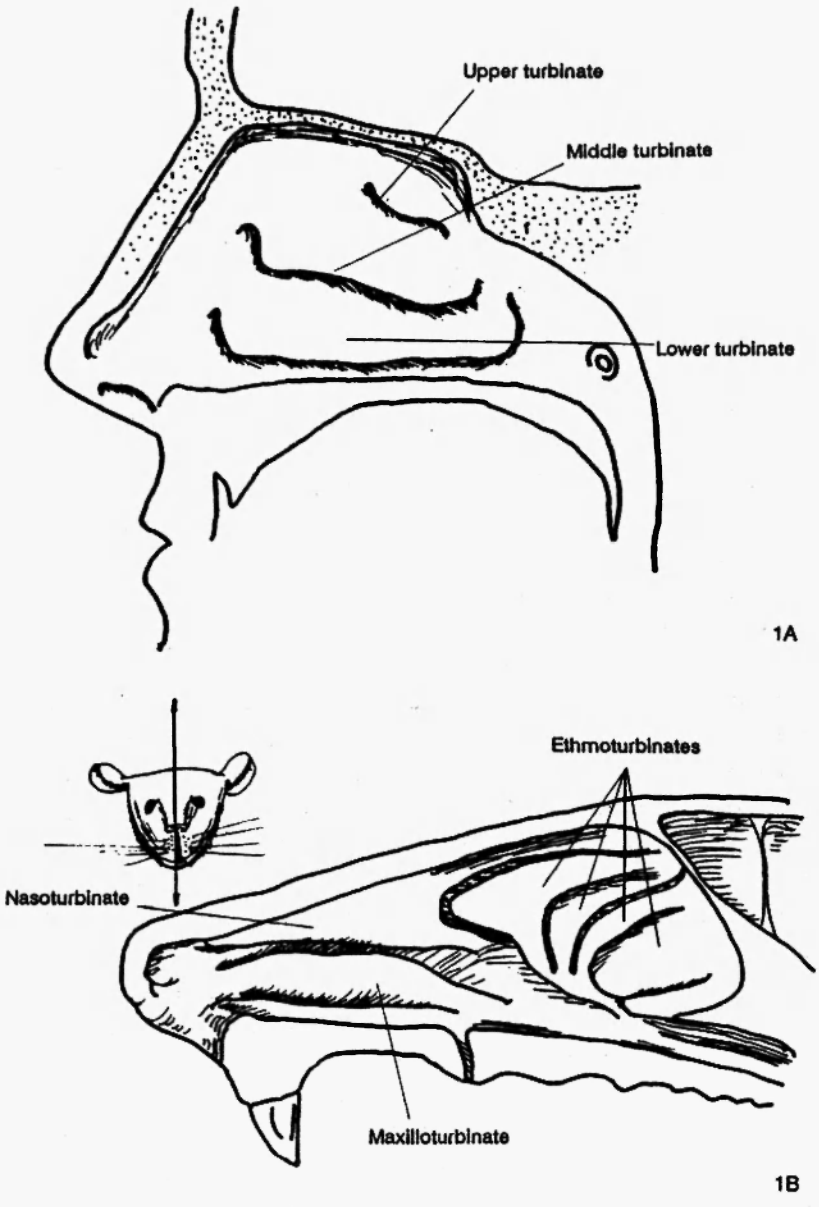
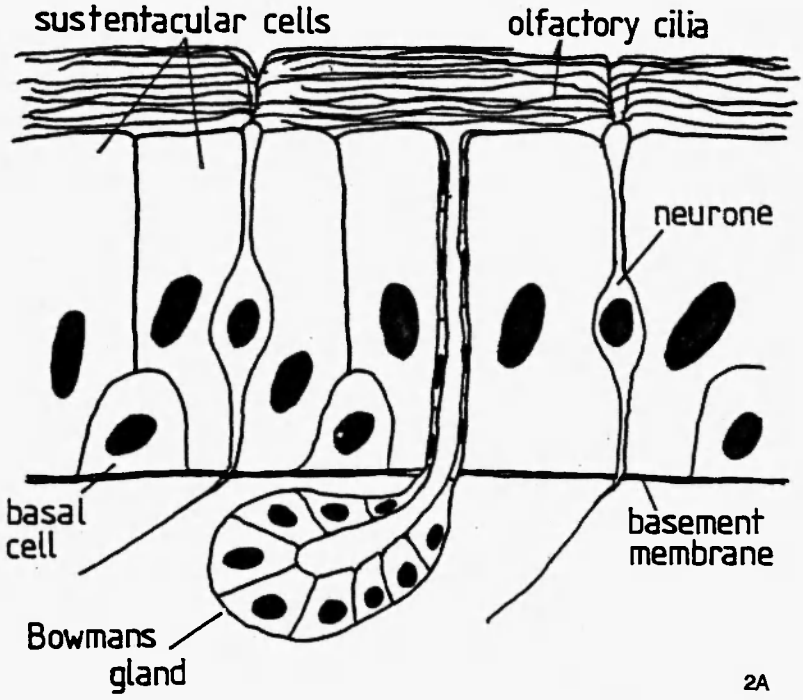


Fig. 1: Diagrams of human (above) and rat (below) nasal passages. Each diagram is of a sagittal section through the head, as illustrated by the inset, showing the lateral wall of the nasal cavities.



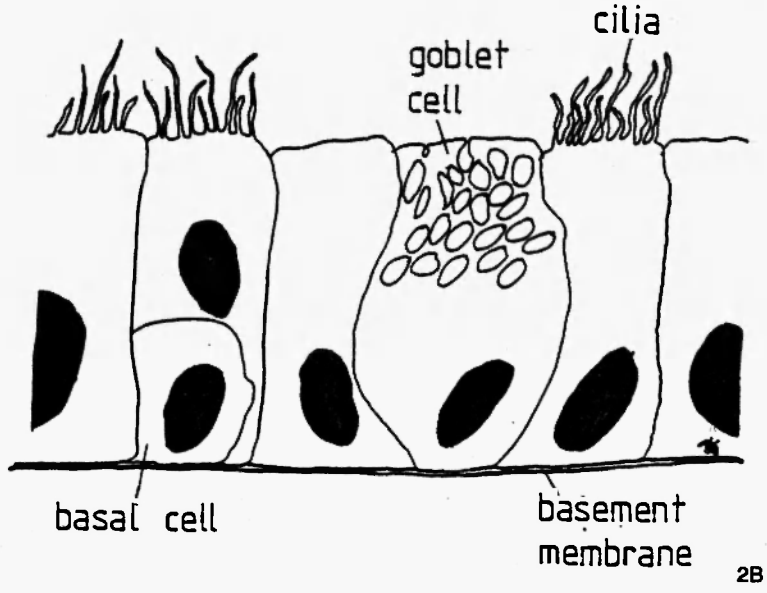


Fig. 2: Diagrams of olfactory (above) and respiratory (below) epithelia (not to scale).

The cell bodies of the olfactory neurones give rise in one direction to the long olfactory cilia which lie in the mucus layer and in the other, to the nerve fibres which pass through the cribriform plate into the olfactory bulb. Respiratory epithelium is a pseudostratified epithelium, consisting of columnar epithelial cells and mitotically active basal cells. Seromucous glands are present below the epithelium and secrete mucus via ducts through the epithelium; mucus is also secreted by the goblet cells in the epithelium itself. In the present paper the terms olfactory epithelium/mucosa and respiratory epithelium/mucosa refer to the specific epithelia, whereas nasal epithelia/mucosae refers to both epithelia together, or studies were distinction between olfactory and respiratory tissue has not been made. In man the surface area covered by the nasal epithelia is approximately 120cm^2, but of this only a few square centimetres, located on the upper part of the upper turbinate, is olfactory epithelium.

Lower animals possess a larger area of olfactory epithelium which is consistent with their increased reliance on the sense of smell for finding food, etc. The anatomy of the nasal passages in animals is different to man in that animals have more turbinates and hence a proportionately larger area of epithelium. Most animals other than man and primates are "obligatory nasal breathers". Due to the anatomy of the nasopharynx in such animals inspired air cannot pass through the mouth but enters the lungs via the nose, thus all inspired chemicals pass over the nasal epithelia. Figure 1 compares the anatomy of the nasal passages in man with those in the rat, commonly used as an experimental animal. In the rat the entire area of the ethmoturbinates is covered in olfactory epithelium which is pigmented. The precise nature of the olfactory pigment is unknown, but it is unique to the olfactory epithelium and as such it is a useful practical identifier of olfactory tissue.

The olfactory epithelium is exposed to all vapours and to particulate aerosols of the correct diameter to impact in the upper respiratory tract. Clearly the particle size of an aerosol, if too small, would prevent the access to the olfactory epithelium of any toxicant they carry. In man particles will start to impact in the nasal passages at about 1μm, at 8μm and above particles impact in the nose /4/. In small animals, however, the decrease in the size of the respiratory

tract results in a decrease in the size of the particles which impact in the nasal passages; in the rat for instance particles of 2μm and above would be expected to impact exclusively in the nasal passages. Such alteration of the impacting particle size will affect the extrapolation of experimental results from animals to man. As a footnote to the above, particles of smaller diameter which impact in the lung may be carried out on the mucus and swallowed.

In recent years many enzyme systems have been identified in nasal epithelia which have the capacity to metabolise a wide variety of xenobiotics. Of the enzyme systems measured in nasal epithelia the cytochrome P-450 monooxygenases have stimulated the most research and are the subject of the next section.

III. NASAL CYTOCHROME P-450

3.1. Distribution among animal species

Cytochrome P-450 isozymes have been found in the nasal tissue of all species so far studied /5,6/. Table 1 shows the levels of the enzyme in some of the species investigated to date. The Syrian golden hamster has the highest amount of P-450 (pmol/mg protein), and the highest *p*-nitroanisole demethylase and aniline hydroxylase activities. High P-450 levels were also found in dogs and rabbits.

TABLE 1

Cytochrome P-450 levels in the nasal mucosa of various species

Species	Cytochrome P-450 (pmol/mg microsomal protein)
Dog (ethmoturbinates)	235± 43 (3)
Rabbit	350± 71 (3)
Guinea Pig	94± 6 (6)
Rat	110± 15 (3)
Syrian Hamster	460± 8 (2)
Mouse	65± 6 (3)

From reference /5/. Values in parenthesis indicate numbers of animals used.

3.2. Location of cytochrome P-450 in nasal tissues.

The location of cytochrome P-450 in the respiratory tract and within the various cell types in tissues is of critical importance when assessing its role in the function of the tissue and in inhalation toxicology.

In large animals such as the dog the various parts of the nasal tissue can readily be separated leaving sufficient material in each part to measure P-450 content and activity. Working with the dog, Dahl *et al* /7/ found the highest content of cytochrome P-450 in the lining of the ethmoturbinates and almost equal amounts in the maxilloturbinates and the nasoepithelial membranes. The P-450 content of the ethmoturbinates was 1/2 to 2/3 that in liver when expressed on an amount/mg protein basis and the content of the lung parenchyma was comparable to that of the maxilloturbinates. When enzymatic activities were compared, however, the *p*-nitroanisole demethylase, aniline hydroxylase and benzo[a]pyrne hydroxylase were found to have higher specific activities in the ethmoturbinates than in liver. Though the presentation of data from three individual dogs used in this study showed considerable inter-individual variation, ratios of enzyme activities and P-450 content were consistent for each individual (Table 2).

TABLE 2

Distribution of Cytochrome P-450 in the Respiratory Tract of the Dog (male Beagles)

Tissue	Dog 1	Dog 2	Dog 3
Nose			
maxilloturbinates	21	42	63
ethmoturbinates	166	262	278
nasal epithelial membranes	19	52	44
Lung			
central parenchyma	10	76	44
subpleural parenchyma	5	5	
Liver	264	362	490

Values are pmol/mg microsomal protein (from reference /7/).

Immunohistochemical studies of the distribution of cytochrome P-450 isoenzymes "c" (major hydrocarbon induced isoenzyme) /8/ and B (phenobarbitone induced) /9/ in olfactory epithelium indicated that they are present in the cells of the Bowmans glands and in sustentacular cells, but not in the olfactory neurones. This location is supported by the proliferation of endoplasmic reticulum in these cells after treatment with agents that induce P-450 (i.e. N-nitrosomethylpiperizine) /10/.

3.3. Characteristics of Nasal Cytochrome P-450

Extrahepatic cytochrome P-450's are seldom investigated without reference to the hepatic enzymes. This is also true of olfactory cytochrome P-450 and some interesting differences between the nasal and hepatic enzymes have been found.

One of the most obvious anomalies posed by olfactory cytochrome P-450 is the high specific activities of some P-450 dependent enzymes compared to those in liver. Recently Reed *et al* /11/ proposed that this was partly due to the high proportion of NADPH:cytochrome P-450 reductase present in olfactory epithelium. The ratio of reductase to cytochrome P-450 in liver was found to be 1:11 to 1:15, whereas that in olfactory microsomes was 1:2 to 1:3. These workers proposed that the increased electron flow from NADPH to the substrate which these high ratios would facilitate, is part of the reason for the high specific activities of the olfactory enzymes. Clearly there are other factors operating in addition to the high reductase concentration since all substrates are not equally affected. The olfactory enzyme, when compared to liver, shows higher specific activities towards some substrates but not to others, as illustrated by the different ratios of hepatic to olfactory demethylase activities found by Hadley and Dahl /12/ and by the higher activity of olfactory P-450 towards 7-ethoxycoumarion but not to 7-methoxycoumarin /13/.

A different isoenzyme composition of the olfactory enzyme would explain the differences in catalytic properties. The hydrocarbon and phenobarbital inducible forms, isoenzymes "c" /8/ and "B" /9/, of cytochrome P-450 have been demonstrated in rat nasal epithelia. In addition Ding *et al*/14/have shown the presence of

the alcohol inducible isoenzyme 3a in rabbit nasal epithelium by immunoblot electrophoresis. These studies were however carried out using antibodies raised to purified hepatic isoenzymes and would not show the presence of isoenzymes peculiar to the olfactory or respiratory mucosae. The existence of such unique isoenzymes, if they exist, remains to be established and must use different methodology.

3.4. Induction and Inhibition of Nasal Cytochrome P-450

Attempts to induce nasal cytochrome P-450 using compounds known to induce hepatic P-450 have been conspicuously unsuccessful. In one such study with the rat, only TCDD (by ip injection) induced nasal aryl hydrocarbon hydroxylase, whereas phenobarbitone, 3-methylcholanthrene, Arochlor 1254, dioxane (all by ip injection and benzo[alpyrene (by inhalation) had no effect /15/. A two fold induction was reported by Hadley *et al* /16/ when phenobarbitone was administered to rats in the drinking water as opposed to by injection, but they could not show induction after inhalational administration of either benzo[a]pyrene or phenobarbitone. Similarly Gillner *et al* /17/ showed two fold inductions of 7-ethoxycoumarin-O-deethylase by β-naphthoflavone which appeared to be associated with an increase in mRNA for P-450d, but not the P-450c induced in liver. Inductions obtained were low when compared to that which can be obtained in liver using the same compounds (between 4 and 10 fold induction dependent upon substrate assayed /18/. Similarly Ding *et al* /14/ could not induce nasal isoenzyme 3a with doses of ethanol which induced the kidney isoenzyme. Brittebo /19/, however, found a two fold induction of aminopyrene demethylase by phenobarbitone but no induction by 3-methylcholanthene after intraperitoneal injection to rats.

In contrast to the apparent failure of classic P-450 inducers, nasal P-450 has been shown to be inhibited by several established P-450 inhibitors, Metyrapone has been consistently shown to inhibit nasal P-450 dependent oxygenations. The dealkylation of phenacetin /20/, aminopyrene /9/, and hydroxylation of benzo[a]pyrene /15/, N'-nitrosonornicotine and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone /21/ in rat nasal tissue are all inhibited by metyrapone.

The P-450 inhibitor proadifen (SKF-525A) also inhibits phenacetin and benzo[a]pyrene metabolism but to a smaller extent than metyrapone at 0.5mM. Investigation of the effect of metyrapone and α-naphthoflavone on the dealkylation of the methoxy- and ethoxy derivatives of coumarin by olfactory P-450 have indicated that more than a simple inhibition is involved /13/. At low concentrations of metyrapone, olfactory deethylase activity was higher than control values. Such "activation might indicate interaction with more than one enzyme, but more detailed kinetic experiments would be required to confirm this.

It has also been demonstrated that the methylene dioxyphenyl perfume and food additives, heliotropin and piperonyl butoxide are capable of inhibiting P-450 in both nasal and hepatic tissues from the rat /22/. Dahl and Brezinski /23/ studied the P-450 interaction of 18 methylene dioxyphenyl compounds. These studies were made using the spectral interactions induced by binding of substrates to microsomal P-450. Analysis of such spectra enables estimates of the amount /24/ and type /18/ of P-450 to be made. Most of these compounds showed a type I spectral interaction with the ferric, oxidised, state of the P-450 (383nm-390nm maximum; 420nm minimum) and a type III spectral change with the ferrous, reduced, P-450 (430nm and 455nm maxima). Further investigation of some of these compounds showed that they were also capable of inhibiting nasal cytochrome P-450 between the concentrations of 5μM and 5mM with I_{50} of between 2μM and 10μM (some calculated by extrapolation).

Recently Petridou-Fischer & Dahl /25/ demonstrated the inhibition of rat and rabbit olfactory hexamethylphosphoramide demethylase and 7-ethoxyresorufin deethylase by a series of dioxalane compounds (Figure 3) which possess a similar methylene function to the methylene dioxyphenyl compounds. Most of the dioxalane derivatives produced a greater inhibition of olfactory P-450 than the hepatic enzyme at equivalent concentrations.

The common occurrence of methylene dioxyphenyl compounds in foods, inhaled air, and as synergists in some insecticides, raises interesting questions with regard to the amount of active nasal P-450. Odorants and flavourings that inhibit or perhaps induce olfactory P-450 will control the level of enzymes available for

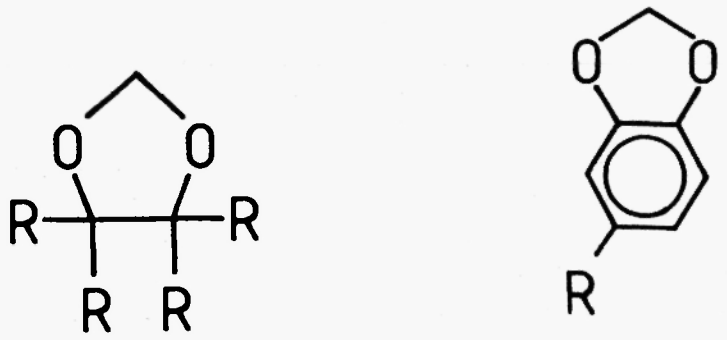


Fig. 3: General formulae of methylene dioxyphenyls and dioxalanes. Note the similarity, a methylene group linked to the rest of the molecule by two ether oxygens.

xenobiotic metabolism, thus placing the emphasis on environmental rather than genetic control of metabolism.

Research has also defined the types of metabolic reactions which nasal cytochrome P-450 and other nasal enzymes can catalyse. The following two sections review some of the oxidative and non-oxidative metabolic reactions demonstrated in the nasal linings.

IV. OXIDATIVE METABOLISM IN NASAL EPITHELIA

4.1. Cytochrome P-450 mediated oxidative demethylation

It has been suggested that one means by which an inhaled chemical carcinogen can operate is via the formation of formaldehyde by cytochrome P-450 mediated oxidative demethylation. Dahl and Hadley /12/ have surveyed some 30 compounds present in inspired air as solvents, pollutants, essences, drugs, and industrial intermediates (some of these compounds are shown in Table 3). Rat nasal microsomes were found to produce formaldehyde at a higher rate than liver microsomes from hexamethylphosphoramide, ethylene diglycol dimethylether, methyal, p-methoxyacetophenone, cocaine, and an extract of diesel exhaust. Lower rates of production by nasal tissue were found for methamphetamine, propylhexidine, pyrilamine, carbinoxamine,

ephedrine, 1-phenylephrine, nicotine, dimethyl anthranilate, dimethylaniline and dimethylsulphate.

TABLE 3

Compounds that are metabolised by nasal cytochrome P-450 by demethylation to produce formaldehyde

Compound type	Examples
Solvents	N,N-dimethylaniline
	Hexamethylphosphoramide
	ethyleneglycol
	Methylal
Air pollutants	Diesel Soot Extract
Essences	Dimethylalanthralite
	p-Methoxyacetophenone
	Methamphetamine
	Propylhexadrine
Nasal Decongestants	Pyrilamine
	Carbinoxamine
Other Drugs	Cocaine
	Nicotine
Dye Intermediates	o,m & *p*-Cresidine

from reference /12/.

4.2. Metabolism of Nitrosamines.

Since the unambiguous demonstration of cytochrome P-450 in nasal mucosa the metabolism of some nitrosamines in this tissue has been extensively investigated. Detailed studies of the metabolism of N-nitrosonornicotine (NNN) and 4-Nitrososamino)-1-(3-pyridyl)-1-butanone (NNK) (Figure 4) were reported by Brittebo *et al.* /26/. These studies performed on rat turbinates in short term organ culture followed the transformations of radiolabelled molecules separated by HPLC. Metabolism of both compounds appeared to be initiated by the hydroxylation of carbon atoms adjacent to the nitroso group nitrogen as indicated in Figure 4. The compounds so formed were unstable and subsequently broke down to form the diazohydroxides thought to be the ultimate

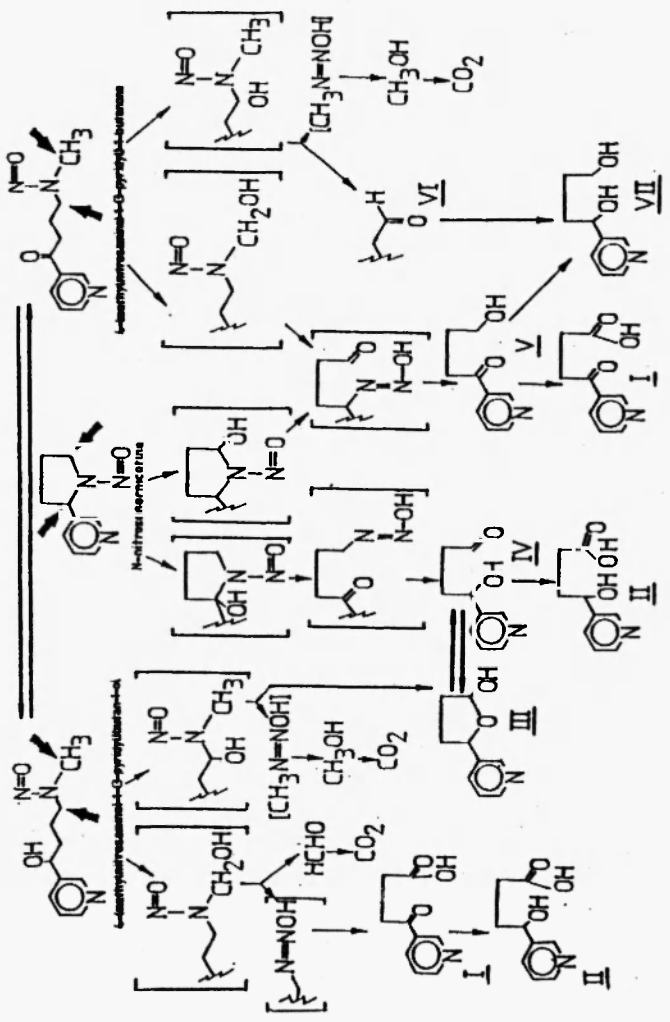


Fig. 4: Metabolism of tobacco specific nitrosamines, N-nitrosonornicotine, 4-(methylnitrosamino)-1-(3-pyridyl)butan-1-ol and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone, by olfactory mucosa. Compiled from reference /26/. Square brackets indicate intermediate compounds. Where only part of a molecule is shown the remainder is unchanged from the parent compound.

carcinogens of nitroamines. Seventy five percent of the radioactivity present in the NNN incubates could be identified as the keto alcohol (*V* in Figure 4), the keto acid (I), and the diol (*VII*). The hydroxy acid (*II*) was also identified. Only 58% of the radioactivity present in the NNK could be identified by HPLC as the α-keto alcohol, keto acid and diol. The carbonyl reduction of NNK yields the 4-(methylnitrosamino)-1-(3-pyridyl)-1-butan-1-ol which by α-hydroxylation produces the hydroxy acid (II).

Brittebo *et al.* /21/ found that N-nitrosopyrrolidine is also metabolised by rat and mouse nasal mucosa. Using data from whole body autoradiographic location of tissue bound metabolites these authors showed liver, kidney and the tracheobronchial mucosa, in addition to the nasal mucosa, were sites of N-nitrosopyrrolidine metabolism. N-nitrosodiethylamine has been found to be deethylated by hamster and rat nasal mucosa with higher activity in the rat /28/. This deethylation could be inhibited by metyrapone, confirming dependence upon cytochrome P-450.

4.3. Oxidation of Polycyclic Aromatic Hydrocarbons

Bond /15/ studied the metabolism of benzo[a]pyrene (BaP) in nasal microsomes from the rat. Metabolites detected by HPLC were BaP-9,10-diol, BaP-4,5-diol, BaP-7,8-diol, BaP-1,6-dione, Ba-P-3,6-dione, 9-hydroxy-BaP and 3-hydroxy-BaP (Figure 5). All but the 7,8-diol were present in both control and TCDD induced animals; the 7,8-diol was only present in the TCDD induced an imals. Moreover Bond and Li /27/ have shown that BaP and 2-aminoanthracene could be converted to Ames test detectable mutagens by the 900 x g supernatant from rat nasal tissue.

It has also been shown that benzo[a]pyrene is metabolised by the olfactory epithelium in dogs after instillation onto the epithelial surface. Petridou-Fischer *et al.* /29/ showed the presence of benzo[a]pyrene tetrols, 9,10-diol, 4,5-diol, 7,8-diol, 9-hydroxide and 3-hydroxide in instillates; indicating that benzo[a]pyrene can cross the mucus and cellular membranes to gain access to the P-450 system.

Given the high activity of olfactory P-450 and the occurrence of chemical carcinogens in the environment a high incidence of

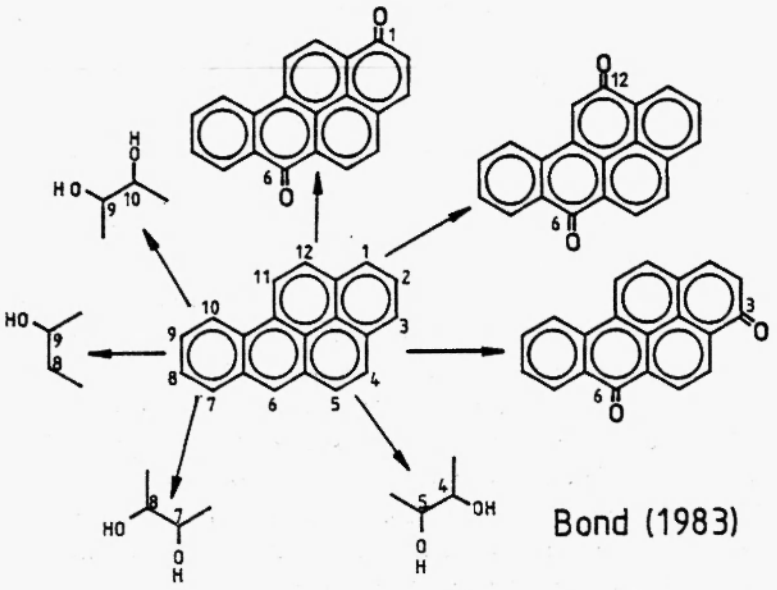


Fig. 5: Oxidative metabolism of benzo[a]pyrene by nasal microsomes. Compiled from reference /15/.

olfactory carcinoma would seem likely. Nasopharyngeal carcinoma is, however, one of the least common carcinomas known. Perhaps the continual movement of mucus over the surface of the epithelium lends some protection from lipid soluble compounds; or the low area of olfactory epithelium in man compared to lower animals reduces the influence of olfactory P-450 on carcinogen metabolism in the nasal cavity. Alternatively, though metabolism of carcinogens in the nasal epithelia of animals is established, evidence that man is also capable of such metabolism is awaited. The absence of critical enzymes or isoenzymes from the nasal epithelia of man might account for low nasopharangeal carcinoma incidence.

4.4. Oxidation of Steroids

Steroids are known to be hydroxylated by cytochrome P-450 dependent oxidases and recently several studies on steroid metabolism in olfactory epithelium have been carried out.

Progesterone has been shown to be metabolised to several products by rat nasal tissue in short term organ culture /30/ (Figure 6). These metabolic products have been separated by TLC, further analysed by gas chromatography/mass spectroscopy and identified as 5-pregnane-3,20-dione (major metabolite), 3-hydroxy-5-pregnane-20-one, 3-hydroxy-5-pregnane-20-one, 20-hydroxy-pregn-4-en-3-one, 6-hydroxy-progesterone, 2,3-dihydroxy-5-pregnane-20-one, 15-hydroxy-progesterone, and 3,16-dihydroxy-5-pregnane-20-one /31/.

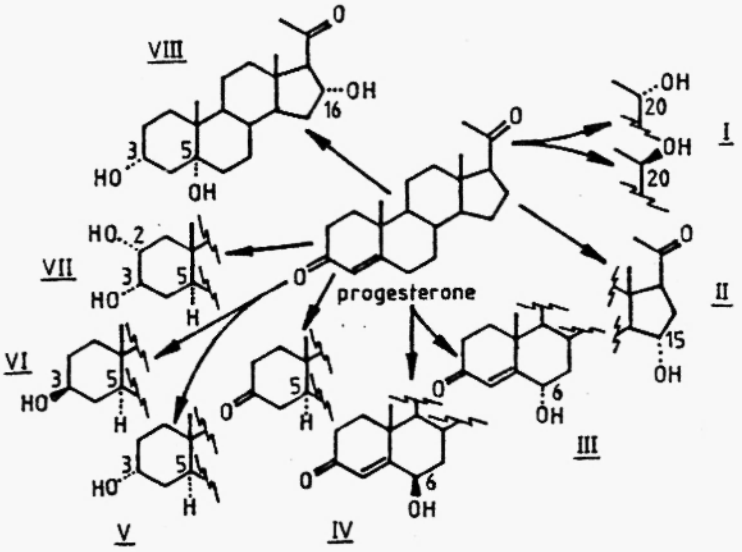


Fig. 6: Metabolism of progesterone by olfactory mucosa. Compiled from reference /31/.

These workers /31/ have also investigated the metabolism of testosterone in rat nasal mucosa (Figure 7). Metabolites isolated were: 4-androstene-3,17-dione, 5-dihydro-testosterone (major metabolite), 3-hydroxy-5-androstane-17-one, 5-androstane-3,17-diol, 2-hydroxytestosterone, 2-hydroxy-05-dihydrotestosterone, 16-hydroxytestosterone, 15-hydroxytestosterone, and 15-androstane-2,3,17-triol. Oestradiol has also been shown to be metabolised to at least three products by rat olfactory mucosa /32/. These metabolites have yet to be conclusively identified.

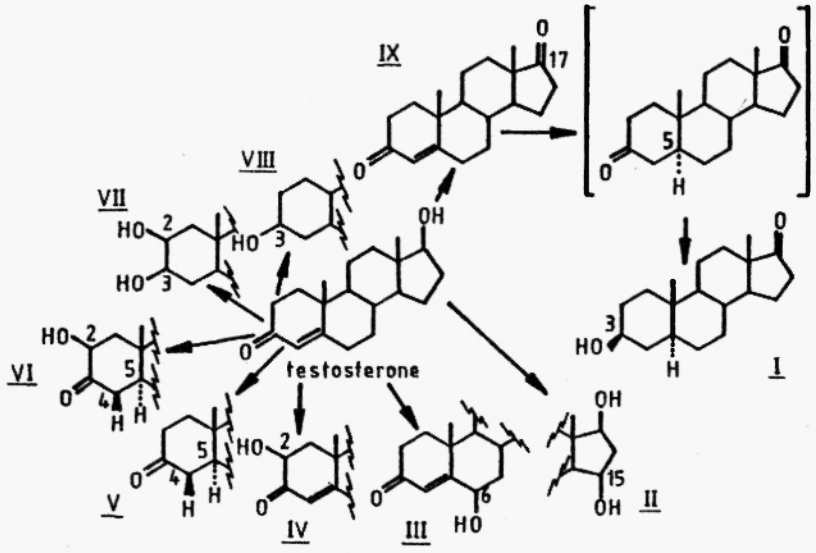


Fig. 7: Metabolism of testosterone by olfactory mucosa. Compiled from reference /31/.

The demonstration of such metabolic products in the olfactory mucosa has led to the suggestion that olfactory tissue contains the enzymes 5α-reductase and 17-hydroxysteroid oxidoreductase. In addition steroid hydroxylases capable of hydroxylation at multiple points on the steroid nucleus are present. It is possible that these hydroxides result from cytochrome P-450 monooxygenase activity since P-450 is known to hydroxylate steroids.

McNulty *et al.* /33,34/ have investigated the demethylation of dimethylamine in Fischer 344 rat nasal tissue. Microsomes from both respiratory and olfactory tissue were able to demethylate dimethylamine, benzphetamine and N,N-dimethylaniline. Demethylation of dimethylamine and N,N-dimethylaniline were resistant to the inhibitory effects of *n*-octylamine at a concentration of 3mM, suggesting the presence in these tissues of different forms of P-450 isozymes. It is likely that these amines may also be metabolised by a flavin-monooxygenase.

V. NON-OXIDATIVE METABOLISM IN NASAL EPITHELIA

As outlined above, P-450 mediated oxidation is often the first step in the metabolism of a foreign compound. Subsequent or concurrent metabolism can be catalysed by a variety of enzymes, some of which have been found in olfactory mucosa.

One study has linked the metabolism of benzo[a]pyrene in rat nasal tissue by oxidative and non-oxidative enzyme systems. Bond /15/ assayed epoxide hydratase with styrene oxide, UDP-glucuronyl transferase with umbelliferone as the acceptor and glutathione transferase with styrene oxide as the acceptor. These enzymes were found to have activities that were 2.5 to 3 orders of magnitude greater than the cytochrome P-450 oxidation of BaP, indicating that the oxidation of this compound may be the rate limiting step in its metabolism. However, since most conjugating enzymes require an energetically activated form of their co-substrate (e.g. UDP-glucuronic acid for glucuronyl transferase and PAP-sulphate for sulphotransferases), it is therefore possible that the tissue levels of these activated precursors limit the activity of these conjugating enzymes.

Olfactory and respiratory epithelia from rats and mice have been found to contain carboxylesterase (*p*-nitrophenylbutyrate substrate), though higher specific activities were found in the olfactory tissue of both species and mice had higher specific activities than rat in both tissues /35/. The esterase was histochemically located (α-naphthylacetate as substrate) in the Bowmans glands and sustentacular cells, but not in the olfactory neurones of olfactory epithelia and in the ciliated respiratory epithelial cells. Non-ciliated respiratory epithelial cells and basal cells stained weakly and goblet cells showed no carboxylesterase activity. Inhibition of α-naphthylacetate esterase in ducts of the Bowmans glands and sustentacular cells with bis-*p*-nitrophenylphosphate and partial inhibition of activity in the Bowmans glands confirms the identity of the esterase as carboxylesterase similar to that which has been well characterised in the liver (for reviews see /36/). All cell types in the respiratory mucosa were inhibited by bis-*p*-nitrophenylphosphate.

In addition to the metabolic production of formaldehyde, nasal tissue can also dehydrogenate formaldehyde and other aldehydes.

Casanova-Schmitz *et al* /37/ have described formaldehyde and acetaldehyde metabolism by NAD^+-dependent dehydrogenases. Both formaldehyde dehydrogenase (EC 1.2.1.1) and two isoenzymes of aldehyde dehydrogenase (EC 1.2.1.3) were found in the respiratory and olfactory epithelia of rats. The specific activities of formaldehyde dehydrogenase and aldehyde dehydrogenase II were higher in homogenates of olfactory epithelia than respiratory epithelia, whereas aldehyde dehydrogenase I was higher in respiratory epithelium. Exposure of animals to formaldehyde vapour did not affect the activities of formaldehyde dehydrogenase in olfactory or respiratory tissue. Exposure to acetaldehyde vapour, however, increased the activities of both isoenzymes of aldehyde dehydrogenase.

VI. METABOLISM MEDIATED TOXICITY IN NASAL EPITHELIA

Several chemicals have recently been shown to have selective toxic effects in nasal epithelia and in particular in the olfactory epithelium. Each of these chemicals is known to be metabolised to a toxic metabolite(s). In each case the ultimate toxic effect is histologically similar, consisting of hyperplasia, atrophy and/or necrosis. After an acute toxic effect, the epithelium can regenerate with the sense of smell apparently unaffected.

Alkyl substitution of the 3rd position of a heterocyclic ring is one common feature between 3-methylfuran, 3-methylindole and 3-(trifluoromethyl)pyridine, all of which are metabolised to toxic metabolites by the nasal epithelia. Whether this structural feature is essential for the nasal toxicities of these compounds is not known. 3-Methylfuran causes necrosis of olfactory epithelium after inhalation; pretreatment with the P-450 inhibitor piperonyl butoxide does not prevent this necrosis /38/. However, necrosis of olfactory epithelium by 3-methylindole could be inhibited by α-naphthoflavone but not by β-naphthoflavone or SKF 525A, indicating the involvement of a P-450 system (probably the P-448 isoenzymes) /39/. 3-(Trifluoromethyl)pyridine causes a dramatic selective necrosis of olfactory epithelium at 1ppm in inhaled air /40/. This necrosis has been linked to metabolic activation of the compound by cytochrome P-450 mediated oxidation /41/.

The metabolism of phenacetin and aminopyrine in nasal tissues have already been cited in other sections of this review. These two xenobiotics are known to be metabolised to toxic metabolites. In rat nasal tissue, bound radioactivity was found after injection of ^{14}C labelled phenacetin and carbon dioxide production could be detected *in vitro* indicating oxidative deethylation /8/. Similar results were obtained when aminopyrine was given to rats and mice, indicating oxidative demethylation /20/ of this compound in nasal tissues.

Carbon tetrachloride metabolites accumulate in nasal epithelia after i.p. injection, and the tissue is capable of producing $^{14}CO_2$ from $^{14}CCl_4$ /42/. In contrast furfural /43/ and methylisocyanate /44/ cause necrotic changes in nasal epithelia but their effects were not experimentally linked to metabolism in the studies cited.

VIII. CONCLUSIONS

That fairly high levels of drug metabolising enzymes, and in particular cytochrome P-450 monooxygenases, are present in olfactory mucosa is now well established. It remains for us fully to assess what compounds are metabolised by them *in vivo* and how this affects the normal function of the tissue. These enzymes are known to be able to metabolise several procarcinogens and other chemicals to their toxic forms. The question that remains unanswered at present is whether these enzymes are capable of metabolising the wide variety of chemicals which the olfactory epithelium is exposed to during the detection of odours.

The role of the nasal epithelia in general is to provide a barrier to the entry of xenobiotics into the organism. The olfactory epithelium, however, has a specialised role in the detection of odours. Almost all chemicals have an odour and must therefore gain access to the olfactory receptors. To the olfactory epithelium the terms "odorant" and "xenobiotic" are synonymous. It would be desirable for odorants to be absorbed into the mucus, interact with the olfactory receptors on the olfactory cilia and immediately diffuse back into the expired air. During transitory "single sniff" olfaction this is probably the predominant method of odorant clearance from the tissue. During chronic exposure, however,

odorant molecules will diffuse into the cells of the epithelium and become subject to metabolism. This might occur in industrial environments where solvents are in use, or in factories making perfumes or flavourings, in addition to the natural odorants present in the environment. Whether it is metabolism or neuronal modulation in the olfactory bulb, or higher centres, which maintain olfactory sensitivity during chronic stimulation, is unknown.

It is perhaps unlikely that high levels of cytochrome P-450 are present in the olfactory mucosa merely to metabolise foreign compounds. If one accepts this argument, then an endogenous compound or compounds must exist as normal substrates for these enzymes. The steroids, prostaglandins, arachidonic acid and fatty acids are known to be substrate for cytochrome P-450 and some of the steroid hormones are already known to be metabolised by olfactory P-450; further research could identify if any more of these compounds are substrates for the enzyme and to what extent this metabolism affects the normal function of the tissue. In animals the susceptibility of individuals to stimulation by pheramones is under hormonal control and the metabolism of steroid hormones in the target tissue is an important element in the mechanism of response to sex hormones. In addition, the localisation of metabolic activities in the Bowmans glands and sustentacular cells of the olfactory epithelium might indicate a possible role in mucus production.

One of the most interesting differences between olfactory P-450 and the hepatic enzyme is the difficulty with which it can be induced and the ease with which it can be inhibited. The reasons for this, and their relevance to the epithelium *in vivo* remain to be assessed, though some authors have suggested that the low apparent inductions are due to the enzyme being already fully induced by chronic exposure to inducers in the environment. The common occurrence of potent inhibitors such as methylene dioxyphenyl compounds in foods, and as synergists in some insecticides, raises interesting questions with regard to the amount of active nasal cytochrome P-450. Xenobiotics which inhibit, or perhaps induce, olfactory P-450 will exercise some control over the level of enzymes available for xenobiotic metabolism, thus placing the emphasis on environmental rather than genetic control of metabolism.

Given the location of the nasal epithelia, their barrier function and the vital role played by the olfactory epithelium in the detection of odours, the linings of the nose will continue to be the subject of active research. In particular the effect of high metabolic capacities on the function of the olfactory epithelium as an organ of special sense remains to be assessed. There has also been recently an interest in using the nasal cavity for systemic drug delivery, particularly for those drugs which suffer extensive first-pass metabolism after oral dosage. Clearly more detailed studies are required to characterise the various drug metabolism enzymes in the nasal tissues in order to assess to what extent drugs are likely to escape metabolism during passage across the nasal epithelia (for review, see /45/).

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