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Intranasal Instillation of Aflatoxin B_1 in Rats: Bioactivation in the Nasal Mucosa and Neuronal Transport to the Olfactory Bulb

Pia Larsson[1] and Hans Tjälve

Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, Swedish University of Agricultural Sciences, Box 573, S-751 23 Uppsala, Sweden

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Aflatoxin B_1 (AFB_1) may be present in moldy dust. Inhalation of contaminated dust particles may result in high local exposure of the nasal mucosa. The present study was designed to assess bioactivation and toxicity of AFB_1 in the nasal mucosa after intranasal administration of the mycotoxin in rats and also to examine if translocation of the mycotoxin occurs from the nasal mucosa to the brain along olfactory neurons. Female Sprague-Dawley rats were given ^{3}H-AFB_1 (0.2, 1 or 20 μg) intranasally and were sacrificed at various intervals (1 h to 20 d). Tissues were examined autoradiographically or histopathologically. Quantitative data were obtained by β-spectrometry in rats given ^{3}H-AFB_1 intranasally or orally (for comparison). The data indicated that intranasal administration of AFB_1 resulted in formation of tissue-bound metabolites in sustentacular cells, in some cells of Bowman's glands, and in a population of neuronal cells in the olfactory mucosa, whereas in the respiratory nasal mucosa, there was selective bioactivation of AFB_1 in mucous cells. Intranasal instillation of 20 μg AFB_1 resulted in disorganized undulating olfactory epithelium, with injured neuronal and sustentacular cells. In the respiratory epithelium, there was selective destruction of mucous cells. β-Spectrometry and autoradiography with tape-sections of the head of rats given ^{3}H-AFB_1 intranasally indicated transport of AFB_1 and/or AFB_1 metabolites along the axons of the primary olfactory neurons to their terminations in the glomeruli of the olfactory bulb. The data indicate that the materials transported in the olfactory nerves represent AFB_1 and/or some of its nonreactive metabolites. It is concluded that application of AFB_1 on the nasal mucosa in rats results in high local bioactivation of the mycotoxin in this tissue and translocation of AFB_1 and/or its metabolites to the olfactory bulb.

Key Words: **aflatoxin B_1; intranasal instillation; olfactory mucosa; nasal respiratory mucosa; olfactory bulb; neuronal transport; bioactivation.**

The toxic and carcinogenic mycotoxin aflatoxin B_1 (AFB_1) is bioactivated by cytochrome P450 enzymes to the AFB_1-8,9-epoxide, which binds to nucleic acids and proteins (Essigmann *et al.,* 1982). Previous studies at our department have shown that several extrahepatic tissues, such as the nasal olfactory and respiratory mucosa, and the mucosa of the trachea and esophagus, in addition to the liver, have a high capacity to bioactivate AFB_1 in various animal species (Larsson *et al.,* 1989, 1994; Larsson and Tjälve, 1992, 1993, 1995, 1996). We have found that the nasal olfactory mucosa had the highest bioactivating capacity in all species.

AFB_1 may contaminate various foods and feeds and may be present in high concentrations in respirable grain-dust particles (Burg and Shotwell, 1984; Sørensen *et al.,* 1981; Selim *et al.,* 1998). Therefore, inhalation of moldy dust may result in high local exposure of the nasal mucosa. The bioactivation of AFB_1 in the nasal mucosa *in vivo* after local exposure of the nasal epithelium has not yet been examined. One objective of the present study was to evaluate bioactivation and toxicity of AFB_1 in the nasal mucosa after intranasal administration of the mycotoxin in rats. For comparison, some rats were also given AFB_1 orally. Tracing of cells with high bioactivating capacity was performed with a microautoradiographic technique, which involved extensive extractions during the fixation and embedding procedures and ensured that all unbound radioactivity would be removed (Larsson and Tjälve, 1992). We also examined the covalent tissue-binding of radioactivity by β-spectrometry. Our previous studies in several animal species have shown that accumulation of firmly bound metabolites of AFB_1 in various tissues *in vivo* is due to local bioactivation of the mycotoxin in these tissues. (Larsson *et al.,* 1989, 1994; Larsson and Tjälve, 1992, 1993, 1995, 1996).

In addition, we examined whether application of AFB_1 on the olfactory epithelium will result in translocation of the mycotoxin to the brain along the olfactory route. This study was based on the observation that the olfactory mucosa poses a possible site of entry of foreign materials into the central nervous system (CNS). Thus, in the olfactory epithelium, the dendrites of the primary olfactory neurons are in contact with the environment in the nasal cavity and, via axonal projections, are also connected with the olfactory bulbs of the brain. It has been shown that some metals and organic xenobiotics can be transported via olfactory neurons (Holl, 1980; Tjälve *et al.,* 1986; Ghantous *et al.,* 1990; Gottofrey and Tjälve, 1991;

[1] To whom all correspondence should be addressed. Fax: +46 (0)18-50 41 44. E-mail: pia.larsson@farmtox.slu.se.

Hastings and Evans, 1991; Tjälve *et al.,* 1996; Henriksson and Tjälve, 1998; Tallkvist *et al.,* 1998).

MATERIALS AND METHODS

Chemicals. [G-^{3}H]Aflatoxin B_1 (^{3}H-AFB_1), dissolved in methanol, with specific activity of 18 Ci (666 GBq)/mmol was obtained from Moravek Biochemicals (Brea, CA, USA). Nonlabeled AFB_1 was purchased from SIGMA Chemical Co. (St. Louis, MO, USA). The isotope solution was evaporated by N_2 gas, after which physiological saline and nonlabeled AFB_1 were added to obtain the concentrations used in different experiments. Other chemicals used in the study were of analytical grade and obtained from regular commercial sources.

Animals. Female Sprague-Dawley rats, body weight about 180 g, were obtained from Bantin & Kingman Universal (Sollentuna, Sweden). The animals were housed at 22°C with 12 h light/dark cycle and given a standard pelleted diet (Lactamin AB, Vadstena, Sweden) and tap water *ad libitum*. The animals were kept for two weeks before the start of the experiments. The studies were approved by the Local Ethics Committee for Animal Research.

Experimental procedures. Rats were given ^{3}H-AFB_1 intranasally. As a means of comparison, some rats were also given the mycotoxin orally. The rats given intranasal instillations were anaesthetized with pentobarbital sodium (40 mg/kg body weight intraperitoneally), and a polyethylene tubing coupled to a Hamilton syringe was inserted into the right nostril until the tubing stopped advancing (about 2 cm). The tubing was then retracted a few millimeters and 10 μl ^{3}H-AFB_1 solution was instilled into the naris. Control rats were given 10 μl physiological saline. The rats dosed orally were given 100 μl ^{3}H-AFB_1 solution into the stomach via a plastic tube attached to a syringe. After varying survival intervals, the rats were killed by CO_2 asphyxiation and used as described below.

Autoradiography with freeze-dried sections of the head was performed in rats injected intranasally with ^{3}H-AFB_1 (50 μCi, 1 μg AFB_1) and killed after 6 h and 24 h (two animals per interval). Autoradiography was then performed according to Ullberg *et al. (*1982), with horizontal tape-sections of the heads. Localization of bound ^{3}H-AFB_1 metabolites in respiratory mucosa, olfactory mucosa, and olfactory bulb was examined by high-resolution microautoradiography according to Larsson & Tjälve (1992) in rats killed 1 h, 6 h, 24 h, 2 d, 5 d, 10 d, 15 d, and 20 d after intranasal administration of ^{3}H-AFB_1 (10 μCi, 0.2 or 20 μg AFB_1). Three animals were used for each treatment and survival interval. The extensive extractions during the fixation and embedding procedures removed all unbound radioactivity and the microautoradiograms therefore showed only tissue-bound labeling (Larson and Tjälve, 1992). Histopathology was performed in rats given the same doses of AFB_1 and killed after 6 h, 24 h, and 5 d (three rats per survival interval). Tissues were fixed in 4% phosphate-buffered formaldehyde solution. Following fixation, the tissues were decalcified in 5.5% EDTA for 2 weeks and embedded in methacrylate (Technovit 7100, Heraus Kulzer, Wehrheim, Germany). Transversal sections 2 μm thick were taken and stained with either hematoxylin-eosin (HE), periodic acid-Schiff (PAS), or toluidine blue.

Quantitative data for the microautoradiography of the olfactory mucosa were obtained by counting silver grains over nuclei and cytoplasms. A scale was used in the ocular of the microscope, which permitted examination of cytoplasmic areas identical to nucleic areas. For each cell type, 10 nuclei and cytoplasms were counted at each survival interval. In the respiratory epithelium, the number of labeled mucous cells were determined by counting labeled cells over a length of 0.3 mm, using a scale in the ocular. For each survival interval, 5 areas were counted.

The amounts of ^{3}H-labeled material in the olfactory nasal mucosa, the olfactory bulb, and the liver in rats killed 6 h and 24 h after intranasal or oral exposure to ^{3}H-AFB_1 (10 μ Ci, 0.2 μg or 20 μg) were determined by β-spectrometry. Total amounts of radioactivity were measured in tissue pieces solubilized in Soluene 350^{TM} (Packard). The amount of tissue-bound radioactivity was determined after extractions of homogenized tissue pieces with 1% SDS and acetone, using the method of Baker and Van Dyke (1984). Extracted protein pellets were dissolved in 1 M NaOH and aliquots were taken for scintillation counting and protein determination according to Lowry *et al.* (1951).

Statistical analysis. Statistical significance was judged with the two-tailed Student's *t*-test for differences between mean values. Statistical analysis of ratios of silver grains over cell nuclei versus cytoplasms or over cell nuclei of darkly stained neuronal cells versus cell nuclei over other cell types were conducted on the logarithms of the data, using the theory of normal distribution. The obtained ratios and their 95% confidence intervals were then antilogarithmated. A difference between cell nuclei and cytoplasms or darkly stained neuronal nuclei and other cell nuclei was considered significant if 1.0 is not included in the 95 % confidence interval.

RESULTS

Autoradiography with Freeze-Dried Sections of the Head

Autoradiography with freeze-dried sections of the heads of rats killed 6 or 24 h after instillation ^{3}H-AFB_1 in the right nostril demonstrated uptake of labeled substance in the right olfactory mucosa. Nerve fascicles beneath the olfactory mucosa, the olfactory nerve layer, and the glomerular layer of the olfactory bulb were labeled. In all rats, this labeling was confined to the medial part of the right bulb (Fig. 1). Labeling was low in the left olfactory mucosa and olfactory bulb.

Microautoradiography of Nasal Olfactory Mucosa

Microautoradiograms of the nasal olfactory mucosa of rats killed 1 h, 6 h, 24 h, and 2 d after intranasal instillation of ^{3}H-AFB_1 showed high labeling in sustentacular cells and in cells of some Bowman's glands (Fig. 2A and B). In the latter cell types, silver grains were more numerous over nuclei than over cytoplasms (Table 1). In sustentacular cells, labeling of the apical portion of the cytoplasm was higher than of the basal portion of the cytoplasm. It was also noted the labeling in Bowman's glands varied; some glands were strongly labeled, whereas others were virtually without silver grains. In addition, there was labeling of neuronal cells, which was lower than in sustentacular cells and labeled cells of Bowman's glands (Table 2). Also, in neuronal cells, labeling of nuclei was higher than labeling of cytoplasms (Table 1).

At 5 and 10 d, there was selective labeling over nuclei of superficially localized neuronal cells with condensed chromatin and darkly stained nuclei (Fig. 2C, Table 1). Labeling of neurons with weakly stained nuclei and sustentacular cells was rather weak at this interval (Table 2). A few Bowman's glands still showed high labeling over nuclei (Fig. 2C, Table 1 and 2). At 15 d and 20 d, the selective labeling of nuclei of darkly stained neuronal cells was very marked (Fig. 2D, Table 1). Thus, these cells were by far the most highly labeled cell population in the olfactory epithelium (Table 2). In the microautoradiograms, there was no detectable labeling of nerve fascicles in olfactory region or olfactory bulb.

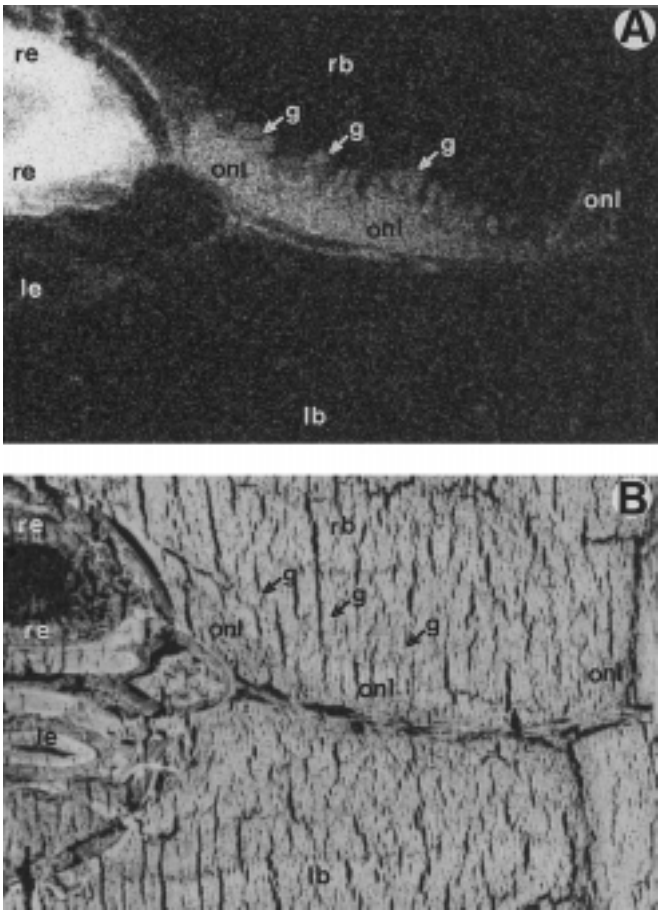

FIG. 1. (A) Autoradiogram (horizontal section) of the head of a rat killed 24 h after instillation of ^{3}H-AFB_1 (50 μCi, 1 μg) in the right nostril. (B) Corresponding freeze-dried tissue-section. Legend: g, glomerulus; lb, left olfactory bulb; le, left olfactory epithelium; onl, olfactory nerve layer; rb, right olfactory bulb; re, right olfactory epithelium. ×20.

Microautoradiography of Nasal Respiratory Mucosa

At all survival intervals (from 1 h to 20 d), there was selective labeling of some mucous cells in the nasal respiratory mucosa (Fig. 3). Labeling was strongest at the shortest intervals. Both cytoplasms and nuclei were labeled. There was a successive decrease in the number of labeled cells from the shortest to the longest survival interval (Table 3). Only a few silver grains were present over ciliated and basal cells in the respiratory epithelium.

Histopathology

Six h after intranasal administration of 0.2 μg AFB_1, the olfactory epithelium appeared morphologically intact. However, in these animals as well as in controls, there were decreased levels of PAS-stainable glycoproteins in Bowman's glands. Six h after intranasal administration of 20 μg AFB_1, the olfactory epithelium was slightly undulating and few cells with pyknotic nuclei were seen in some areas. Respiratory epithelium appeared morphologically intact in both groups of rats at 6 h.

Twenty-four h after intranasal instillation of 20 μg AFB_1, the olfactory epithelium was disorganized and had an undulating appearance (Fig. 4B). Several cells with pyknotic nuclei and shrinked cytoplasms were present. Both sustentacular and neuronal cells were injured, whereas cells of Bowman's glands appeared morphologically intact. Although less marked, similar injury was seen in rats given 0.2 μg mycotoxin. The extension of injured areas was usually most marked at dorsal parts of the nasal septum and medial parts of endoturbinates. In control rats (given saline), there were no injuries in the olfactory epithelium at the 24 h interval (Fig. 4A). However, there was still decreased staining of glycoproteins in Bowman's glands at the site of intranasal instillation. Respiratory epithelium was largely intact 24 h after instillation of 0.2 μg AFB_1. However, after instillation of 20 μg AFB_1, mucous cells in some areas of the epithelium were severely damaged with pyknotic nuclei and vacuolized cytoplasms. Ciliated and basal cells appeared largely intact (Fig. 5).

Five days after AFB_1 exposure, some areas of the nasal olfactory and respiratory epithelium were still damaged in rats given 20 μg mycotoxin. However, the extent of damaged areas was smaller than at 24 h. In rats given 0.2 μg AFB_1, both olfactory and respiratory epithelium appeared intact at 5d.

β-Spectrometry

The amount of covalently bound ^{3}H-AFB_1 metabolites in the nasal olfactory mucosa was much higher than in the liver, both at 6 h and 24 h after intranasal instillation of the mycotoxin (Table 4). In the two groups of animals exposed orally to ^{3}H-AFB_1 and killed after 6 h, higher levels of covalently bound ^{3}H-AFB_1 metabolites were found in the liver than in the olfactory mucosa. Similar amounts of bound ^{3}H-AFB_1 metabolites were found in liver and olfactory mucosa in the group of animals killed 24 h after oral administration.

β-Spectrometry performed in animals instilled with ^{3}H-AFB_1 in the right nostril showed that there was an uptake of labeled material in the right olfactory bulb at both survival intervals, which exceeded the uptake in the left bulb by about 4–6 times (Table 5).

DISCUSSION

The data reported in the present study indicate that intranasal instillation of AFB_1 results in bioactivation of the mycotoxin in cells of Bowman's glands, sustentacular cells, and a population of neuronal cells. The observation that cells of Bowman's glands and sustentacular cells have a high capacity to bioactivate AFB_1 correlates with observations in rats and several other species given the mycotoxin systemically or in which nasal tissue was incubated with ^{3}H-AFB_1 (Larsson *et al.*, 1989, 1994; Larsson and Tjälve, 1992, 1995, 1996). The observation

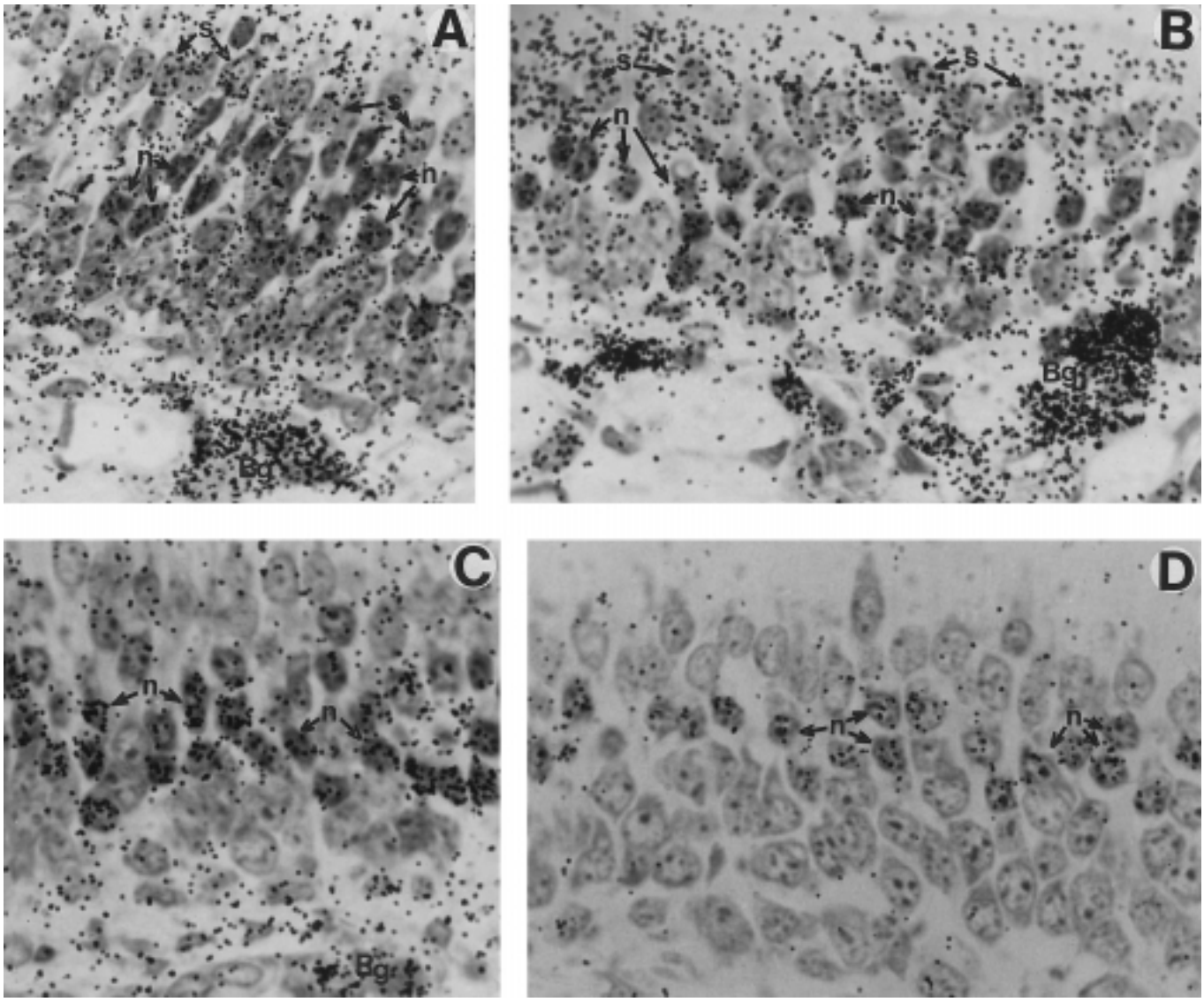

FIG. 2. Microautoradiograms of the right olfactory mucosa of rats killed 6 h (A), 24 h (B), 10 d (C), and 15 d (D) after instillation of ^{3}H-AFB$_1$ (10 μCi, 0.2 μg) in the right nostril. Legend: Bg, cells of Bowman's glands; n, neuronal cells; s, sustentacular cells. Toluidine staining; ×360.

that AFB$_1$ is bioactivated in a population of neuronal cells has not been described before. The bioactivation is catalyzed by several cytochrome P450-enzymes (Putt *et al.,* 1995; Gallagher *et al.,* 1996; Béréziat *et al.,* 1995), and these enzymes are primarily localized in Bowman's glands and sustentacular cells (Thornton-Manning and Dahl, 1997; Voigt *et al.,* 1993). On the other hand, it has been reported that the levels of cytochrome P450 enzymes are low in olfactory neurons (Thornton-Manning and Dahl, 1997). However, it is known that AFB$_1$ can also be bioactivated by prostaglandin H synthase and lipoxygenase (Amstad and Cerutti, 1983; Liu and Massey, 1992; Donnelly *et al.,* 1996), and these enzymes may potentially be responsible for neuronal bioactivation of AFB$_1$ in olfactory neurons. Prostaglandin H synthase and lipoxygenase are present in neuronal tissues (Piomelli, 1994), but, to our knowledge, their possible occurrence in olfactory neurons has not been examined.

The neuronal cell population which accumulated bound ^{3}H-AFB$_1$ labeling were present superficially in the epithelium and showed darkly stained nuclei. It can be assumed that these neurons are the more mature ones, since they are characterized by a more condensed chromatin than basally localized immature neurons (Schwob *et al.* 1992).

In all cell types of the olfactory epithelium, there was preferential localization of silver grains over nuclei. We have previously shown nuclear localization of bound AFB$_1$ metabolites in cells with a capacity to bioactivate the mycotoxin (Larsson *et al.,* 1989, 1994; Larsson and Tjälve, 1992, 1995, 1996). It has been reported that the AFB$_1$ epoxide readily binds

TABLE 1
Ratios of Number of Silver Grains over Nuclei Versus Cytoplasms in Different Cell Types in Microautoradiograms of Rat Nasal Olfactory Mucosa

Survival interval	Darkly stained neuronal cells	Weakly stained neuronal cells	Sustentacular cells	Cells of Bowman's glands
6 h	1.8 (1.6–2.1)[a]	1.8 (1.5–2.1)[a]	1.9 (1.6–2.3)[a]	2.0 (1.8–2.3)[a]
24 h	2.5 (2.1–3.0)[a]	2.1 (1.7–2.8)[a]	1.2 (1.0–1.5)	3.6 (2.8–4.6)[a]
10 d	9.7 (7.6–12.4)[a]	1.5 (1.1–1.9)[a]	1.4 (0.9–2.3)	5.1 (3.5–7.4)[a]
15 d	14.3 (11.3–18.2)[a]	2.3 (1.6–3.3)[a]	1.8 (1.3–2.7)[a]	2.6 (1.5–4.4)[a]

Note. Rats were instilled with ^{3}H-AFB_1 (10 μCi, 0.2 μg) in the right nostril. Values shown are the ratio of the mean number of silver grains over nuclei versus cytoplasms of the different cell types. For each cell type, 10 nuclei and cytoplasms were counted at each survival interval.

[a]Significantly different labeling between nucleus and cytoplasm; 1.0 is not included in the 95% confidence interval.

to double-stranded DNA (Yu *et al.*, 1990), and labeling of nuclei probably reflects a specific affinity to bioactivated AFB_1 for DNA.

It appeared that at long survival intervals, there was retention of bound ^{3}H-AFB_1 labeling in neuronal nuclei, which was more apparent than in other mucosal cells. The reason for this is not known, but one possibility is that repair of adducted DNA occurs at slower rate in neuronal cells than in other cells. The AFB_1-8,9-epoxide binds to DNA primarily at the N^7 position of guanine (Essigman *et al.*, 1977; Lin *et al.*, 1977). Altered guanine bases are either removed enzymatically or spontaneously at the glycosidic bond (Croy and Wogan, 1981; Loeb, 1985). It is conceivable that enzymatic adduct removal occurs at a slow rate in neuronal cells.

The finding that there was high labeling of some Bowman's glands, whereas others were virtually devoid of radioactivity, may be related to variations in contents of different cytochrome P450 enzymes in these glands. Bowman's glands present in the olfactory mucosa of the dorsal medial meatus of the nasal cavity contain higher levels of some cytochrome P450 enzymes than Bowman's glands at other sites (Genter *et al.*, 1995). Our observation indicated that there may also be variations in cytochrome P450 contents in Bowman's glands within the same areas of the olfactory mucosa.

TABLE 2
Ratios of Number of Silver Grains over Nuclei in Darkly Stained Neuronal Cells Versus Nuclei in Other Cell Types in Microautoradiograms of Rat Nasal Olfactory Mucosa

Survival interval	Darkly stained/ weakly stained neuronal cells	Darkly stained neuronal cells/ sustentacular cells	Darkly stained neuronal cells/ Bowman's cell glands
6 h	1.0 (0.9–1.1)	0.5 (0.4–0.6)[a]	0.4 (0.4–0.6)[a]
24 h	1.4 (1.1–1.7)[a]	0.6 (0.5–0.7)[a]	0.5 (0.4–0.6)[a]
10 d	8.1 (5.5–11.9)[a]	5.1 (3.5–7.5)[a]	1.3 (0.9–1.9)
15 d	6.8 (4.6–10.1)[a]	6.8 (4.6–10.0)[a]	3.7 (2.5–5.6)[a]

Note. Rats were instilled with ^{3}H-AFB_1 (10 μCi, 0.2 μg) in the right nostril. Values shown are the ratio of the mean number of silver grains over nuclei of the darkly stained neuronal cells versus the mean number of silver grains over nuclei of the other cell types. For each cell type, 10 nuclei were counted at each survival interval.

[a]Significantly different labeling between nuclei of darkly stained neuronal cells and nuclei of other cell types; 1.0 is not included in the 95% confidence interval.

Our results indicated potent bioactivation of AFB_1 in mucous cells in the nasal respiratory mucosa. The successive decrease in number of labeled mucous cells at increasing survival intervals may reflect the life span of these cells. The mucous cells have previously been shown to bioactivate other xenobiotics, such as N-nitrosodiethylamine and ipomeanol (Reznik-Schüller, 1982; Larsson and Tjälve, 1988). We have previously shown high bioactivation of AFB_1 in respiratory mucosa of several animal species (Larsson and Tjälve, 1992, 1993, 1996; Larsson *et al.*, 1994).

We assume that the toxic effect of AFB_1 in the nasal mucosa is related to bioactivation of the mycotoxin. Bioactivation-related toxicity of AFB_1 in tracheal mucosa has been observed following intratracheal instillation of AFB_1 in rabbits (Coulombe *et al.*, 1986).

A part of the intranasally administered AFB_1 can be expected to be absorbed into the blood either locally in the nasal area or after dislocation to the respiratory and alimentary tract followed by uptake to circulation. Indeed, bound AFB_1 metabolites were found in the liver at similar levels after intranasal and oral administration of the mycotoxin.

Our data showed that ^{3}H-AFB_1 radioactivity was localized in the olfactory bulb ipsilateral to the site of administration. Since labeling was observed in olfactory nerve fascicles beneath the olfactory mucosa and in the olfactory nerve layer of the olfactory bulb at the side of application, and since labeling and was low in the contralateral bulb, we may assume that the radioactive material is transported along the axons of the olfactory receptor cells. Since our data indicated metabolism of AFB_1 in a population of olfactory neurons, we may presume that the transported material is formed, at least in part, within the neuronal cells.

The observation that labeling reached the glomeruli of the olfactory bulb, in which synapses between primary and

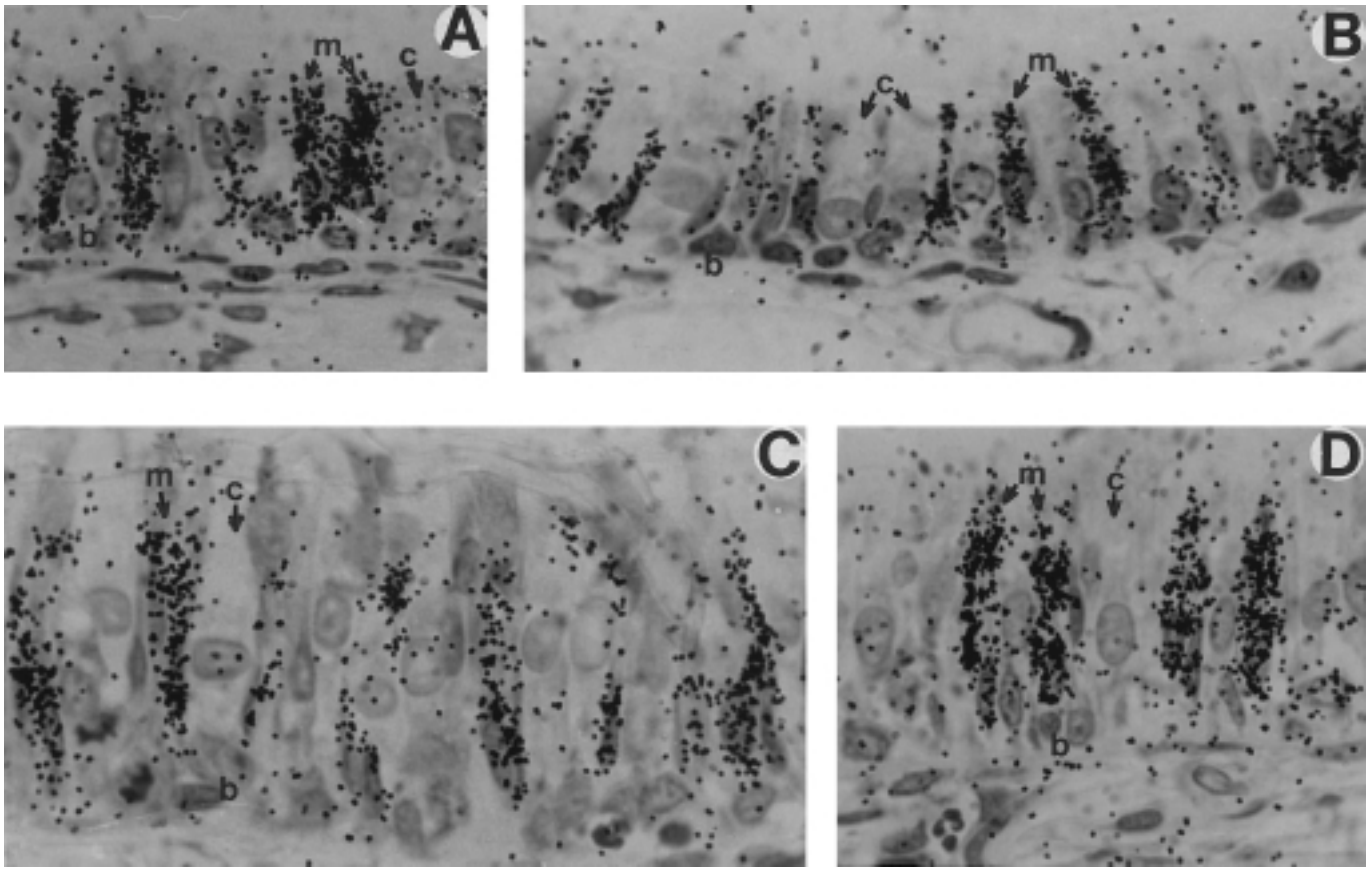

FIG. 3. Microautoradiograms of the right nasal respiratory mucosa of rats killed 1 h (A), 24 h (B), 2 d (C), and 5 d (D) after instillation of ^{3}H-AFB$_1$ (10 μCi, 0.2 μg) in the right nostril. Legend: b, basal cells; c, ciliated cells; m, mucous cells. Toluidine staining; ×360.

secondary olfactory neurons are localized, indicated that the transported material reached the terminals of the axons of primary olfactory neurons but was unable to pass to secondary olfactory neurons. The identity of the transported material is not known. However, the microautoradiography, which in the present study included fixation and embedding procedures and therefore will show only tissue-bound radioactivity (Larsson and Tjälve, 1992), demonstrated lack of labeling of olfactory nerve fascicles beneath the olfactory mucosa and of the nerve layer of the olfactory bulb. Thus, the material in the olfactory nerves should represent AFB$_1$ and/or some of its nonreactive metabolites. The latter constitute various hydroxylated AFB$_1$ metabolites and AFB$_1$ conjugates. It is possible that the labeled material within the olfactory nerves will adhere to and move with some cellular components undergoing axoplasmic transport. Alternatively, movement of the labeled material may be due to diffusion along the olfactory axons.

TABLE 3
Number of Labeled Mucous Cells in Microautoradiograms of Rat Nasal Respiratory Mucosa

Survival interval	Number of labeled cells/ mm of respiratory mucosa[a]
1 h	60.8 ± 11.9
24 h	51.9 ± 6.2
2 d	44.4 ± 12.6
5 d	26.5 ± 5.9
10 d	15.9 ± 5.0
15 d	14.3 ± 3.6
20 d	6.9 ± 4.8

Note. Rats were instilled with ^{3}H-AFB$_1$ (10 μCi, 0.2 μg) in the right nostril. Values are expressed as mean ± SD; n = 5.

[a] The number of labeled cells were determined in five different areas of nasal respiratory mucosa, each comprising a length of 0.3 mm.

Local exposure of nasal mucosa to AFB$_1$ may induce tumorigenesis of this tissue. As mentioned, high levels of AFB$_1$ can be present in respiratory grain-dust particles (Sørensen *et al.*, 1981; Burg and Shotwell, 1984; Selim *et al.*, 1998), which may lead to high local exposure of the nasal mucosa, and may conceivably increase risk for tumorigenesis of this tissue. Tumors originating from nasal mucosa are rare in humans (Silva *et al.*, 1983). However, in an epidemiological study in Dutch

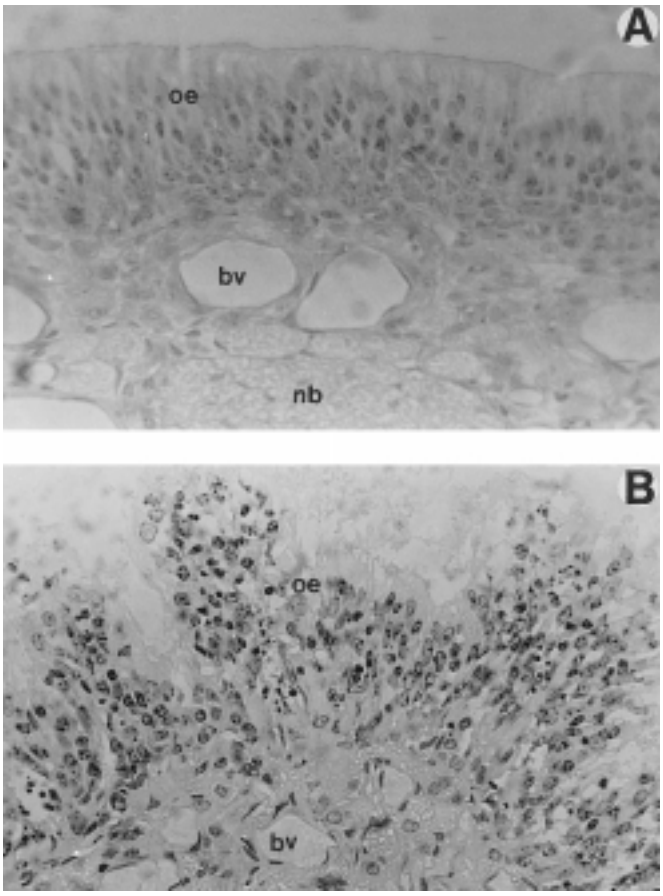

FIG. 4. Sections of nasal olfactory mucosa at the right dorsal meatus of rats killed 24 h after instillation of (A) physiological saline (control) or (B) 20 μg AFB_1 in the right nostril. Legend: bv, blood vessel; nb, nerve bundles; oe, olfactory epithelium. PAS staining; ×115.

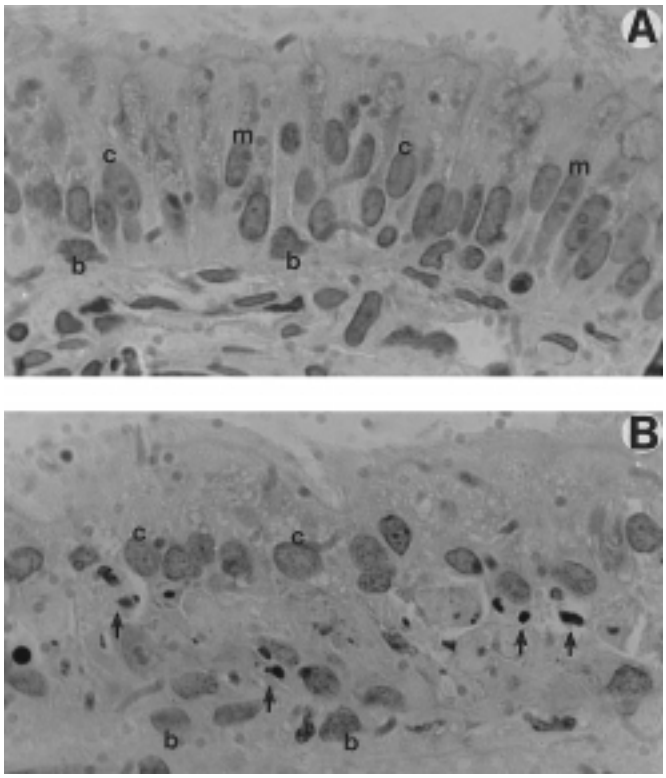

FIG. 5. Sections of nasal respiratory mucosa at the right side of the nasal septum of rats killed 24 h after instillation of (A) physiological saline (control) or (B) 20 μg AFB_1 in the right nostril. Arrows indicate pyknotic nuclei of mucous cells; b, basal cells; c, ciliated cells; m, mucous cells. HE staining; ×270.

oil press workers industrially exposed to aflatoxins by inhalation, one case of nasal cancer was observed among 11 cancers in a group of 55 workers (Hayes *et al.,* 1984). Domestic animals, such as cattle, sheep, and swine, which are exposed to AFB_1 via heavily contaminated feed and thus may inhale high amounts of the mycotoxin, have shown increased incidence of tumors originating from the nasal mucosa (Rajan *et al.,* 1972; Pospischil *et al.,* 1979; Sreekumaran and Rajan, 1983). Nasal tumors have also been observed in sheep experimentally exposed to AFB_1-contaminated groundnut feed (Lewis *et al.,* 1967).

Our observation that AFB_1 appears to be bioactivated in some neuronal cells in the olfactory epithelium indicates that these cells may undergo tumorigenesis. It can be mentioned that in a carcinogenicity study in monkeys given AFB_1 intraperitoneally or orally, one case of olfactory neuro-epithelioma was observed among nine cancers in a group of 45 animals (Adamson and Sieber, 1979).

To our knowledge, there is no evidence that AFB_1 may induce tumors in olfactory bulbs. Thus, while tumors originating from nasal mucosa are frequently found in livestock exposed to moldy feed, no such evidence exists for forebrain neoplasms. The lack of CNS carcinogenesis is likely due to the inability of AFB_1 to pass from primary olfactory neurons to secondary or other neuronal connections in the olfactory system.

TABLE 4
Amount of Covalently Bound AFB_1 Metabolites in Nasal Olfactory Mucosa and Liver of Rats

		Intranasal instillation		Oral administration	
Survival interval (h)	Dose (μg)	Olfactory mucosa	Liver	Olfactory mucosa	Liver
6	0.2	2289 ± 133[a]	11 ± 3	2 ± 1[b]	28 ± 9
6	20	36615 ± 13108[a]	403 ± 212	40 ± 28[b]	242 ± 47
24	0.2	2908 ± 339[a]	16 ± 6	8 ± 9	18 ± 3

Note. Rats were exposed intranasally or orally to ^{3}H-AFB_1 (10 μCi). Values are expressed as mean ± SD; n = 5. Covalent binding is expressed in pmol/mg protein.

[a] Significantly different from livers of intranasally instilled rats, $p < 0.005$.
[b] Significantly different from livers of orally administred rats, $p < 0.005$.

TABLE 5
Amount of Labeled Material (AFB_1 and/or AFB_1 Metabolites) in Olfactory Bulbs of Rats

Survival interval (h)	Right olfactory bulb	Left olfactory bulb	Ratio right/left
6	1.3 ± 0.7^a	0.4 ± 0.2	3.6 ± 1.2
24	1.2 ± 0.3^b	0.3 ± 0.1	6.3 ± 4.2

Note. Rats were instilled with ^{3}H-AFB_1 (10 μCi, 0.2 μg) in the right nostril. Values are expressed as mean $\pm$ SD; n = 5. Amount of labeled material is expressed in pmol/mg tissue.

[a]Significantly different from left olfactory bulb, $p < 0.05$.

[b]Significantly different from left olfactory bulb, $p < 0.001$.

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