Carcinogenesis vol.10 no.6 pp.1113–1118, 1989

1-230 ROGER A. COULOMBE

Metabolism of aflatoxin B_1 in the bovine olfactory mucosa

Pia Larsson, Hans Pettersson[1] and Hans Tjälve

Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, the Swedish University of Agricultural Sciences, Uppsala Biomedical Center, Box 573, S-751 23 Uppsala, and [1]Department of Animal Nutrition and Management, Faculty of Agriculture, the Swedish University of Agricultural Sciences, Box 7046, S-750 07 Uppsala, Sweden

Carcinomas of the ethmoidal region of the nose are observed relatively frequently in cattle in several countries in tropical and subtropical latitudes. Viruses have been implicated as causative agents, but it has been observed that affected animals sometimes suffer from aflatoxicosis, and a role of aflatoxin B_1 (AFB_1) in the aetiology has also been proposed. We have examined whether the bovine nasal olfactory mucosa has a capacity to metabolize AFB_1. The contents of cytochrome P-450 and cytochrome b_5, and the NADPH cytochrome *c* reductase activity in the nasal olfactory mucosa have also been determined. Comparative experiments have been performed with the liver. Incubations with ^{3}H-labelled AFB_1 showed that the nasal olfactory mucosa has a much higher capacity than the liver to form lipid-soluble, water-soluble and tissue-bound AFB_1-metabolites. High-resolution microautoradiography showed a strong localization of tissue-bound metabolites in the sustentacular cells in the apical portion of the olfactory surface epithelium and in Bowman's glands in the olfactory lamina propria mucosae. Especially in the sustentacular cells the labelling was preferentially located in the nuclei of the cells. Liquid chromatography of chloroform extracts of the nasal olfactory mucosa and the liver incubated with ^{3}H-AFB_1 showed formation of several metabolites. The dominating peak in both tissues was aflatoxin M_1 (AFM_1). However, the amount of AFM_1 was higher in the nasal olfactory mucosa than in the liver, and the amounts and proportions of several other metabolites also differed markedly between the two tissues. The level of cytochrome P-450 in the nasal olfactory mucosa was found to be about one quarter of that in the liver, but the NADPH cytochrome *c* reductase activity was much higher in the nasal olfactory mucosa than in the liver. In addition, the cytochrome b_5: cytochrome P-450 ratio was higher in the nasal olfactory mucosa than in the liver. The higher metabolism of AFB_1 in the nasal olfactory mucosa than in the liver may be related to differences in the cytochrome P-450 isoenzyme profile. In addition, the microsomal electron transport to cytochrome P-450 may be facilitated by the high reductase: cytochrome P-450 ratio and the high cytochrome b_5: cytochrome P-450 ratio in the nasal olfactory mucosa. It is considered that the results of the present study strengthen the hypothesis that exposure of AFB_1-contaminated feed may be an important aetiological factor in the development of nasal tumours in cattle.

*__Abbreviations:__ AFB_1, aflatoxin B_1; AFB β_{2a}, aflatoxin B_{2a}; AFM_1, aflatoxin M_1; AFP_1, aflatoxin P_1; AFQ, aflatoxin Q_1; AFL aflatoxicol; ^{3}H-AFB_1, (G-^{3}H)-aflatoxin B_1; LC, liquid chromatography; PAS, periodic acid Schiff.

Introduction

Aflatoxins are mycotoxins produced by fungi of the species *Aspergillus flavus* and *A.parasiticus*. Cattle and other farm animals are exposed to aflatoxins primarily via contaminated cereals, molasses and other concentrated feed-stuffs. The toxin production is favoured by warm and moist environments and aflatoxin contamination is therefore most common in tropical and subtropical latitudes (1,2).

Clinical signs of chronic aflatoxin-intoxication in cattle are rather non-specific and include anorexia, diarrhoea, impaired growth and reduced milk production (2,4). Liver function is impaired and hepatic lesions, such as bile duct hyperplasia and periportal fibrosis, can be observed at postmortem examinations (3,4). Aflatoxin-induced liver tumours have been observed in several species of experimental animals and epidemiological studies have shown a positive correlation between liver cancer in man and high levels of aflatoxin contamination in some areas of the world (1,5). However, liver cancer has not been reported to be a detectable natural phenomenon in cattle.

Carcinomas of the ethmoidal region of the nose are observed in cattle in several developing countries (6–11). In some areas of India the incidence of these tumours has risen and reached endemic proportions (11). An exceptionally high incidence has also been reported in the Dominican Republic (9).

The cause of the bovine ethmoidal carcinoma is not known, but it has been proposed that a virus may be involved (11). It has been observed that affected animals sometimes suffer from severe aflatoxicosis and a role of aflatoxins in the aetiology has also been suggested (9).

It has been shown that aflatoxin B_1 (AFB_1*) may be present in high concentrations in respirable grain dusts (12,13). Conceivably, cattle may inhale AFB_1-contaminated particles leading to a local exposure of the olfactory mucosa and this phenomenon may increase the risk of tumour induction.

There are some indications in other species that exposure to aflatoxins may be causally connected with nasal tumours. Thus, among seven sheep given aflatoxin contaminated groundnut feed two animals developed ethmoidal tumours, whereas in one animal a liver carcinoma was observed (14). Among 45 monkeys given AFB_1 one case of a nasal olfactory tumour was observed, in addition to nine liver carcinomas and single cases of haemangio-endotheliomas of the liver and the pancreas (15). Eight cases of nasal cavity tumours were observed in rats, which had been exposed to AFB_1 transplacentally or during early post-natal life (in this study a total of 197 tumours were observed in 483 test rats) (16). In an epidemiological study in Dutch oil press workers, industrially exposed to aflatoxins, one case of nasal cancer was observed among 11 cancers in a group of 55 workers (17).

AFB_1, which is the most potent carcinogen among the aflatoxins produced by the *Aspergillus* fungi, is a procarcinogen requiring metabolic activation to exert its carcinogenic effects. Many metabolites of AFB_1 are known and the AFB_1-8,9-exposide-intermediate has been postulated to be the ultimate carcinogenic form, which binds to macromolecules, including

© IRL Press

Table I. Contents of cytochrome P-450 and cytochrome b_5 and NADPH cytochrome *c* reductase activity in microsomal preparations of bovine olfactory mucosa and liver (mean ± SD of six/for cytochrome P-450/or four/for cytochrome b_5 and NADPH cytochrome *c* reductase/determinations)

	Olfactory mucosa	Liver
Cytochrome P-450 (nmol/mg protein)	0.28 ± 0.06	1.08 ± 0.2
Cytochrome b_5 (nmol/mg protein)	0.35 ± 0.04	0.95 ± 0.08
NADPH cytochrome *c* reductase (nmol cytochrome *c* reduced/min., mg protein)	342 ± 37	66 ± 9

All values for the olfactory mucosa were significantly different from those of the liver ($P < 0.001$).

DNA (1). The activation of AFB_1 appears to be carried out by the cytochrome P-450 system (1). It has been shown that the nasal olfactory mucosa of a number of species contains cytochrome P-450 and is capable of metabolizing many xenobiotics (18–21).

In the present study, we have examined whether AFB_1 is metabolized in the bovine nasal olfactory mucosa. In the experiments the olfactory mucosa was incubated with ^3H-labelled AFB_1. The formation of unbound and tissue-bound metabolites was then determined by liquid chromatography (LC) and liquid scintillation counting. In addition, the localization of tissue-bound ^3H-AFB_1-metabolites was examined in specimens of the nasal olfactory mucosa by high-resolution microautoradiography. Since the level of cytochrome P-450 in the bovine olfactory mucosa has not been reported, we performed such a determination. In addition, we examined the NADPH cytochrome *c* reductase activity and the contents of cytochrome b_5 in the olfactory mucosa. There is evidence that the provision of electrons to cytochrome P-450 may be rate-limiting in the catalytic circle (21) and it is of interest therefore to examine the components of the associated electron transport systems. The first of the two electrons required is donated by NADPH, whereas the second one may be derived either from NADPH or from NADH via cytochrome b_5 (22).

In our study comparative experiments have been performed with bovine liver.

Materials and methods

Chemicals

(G-^3H)-aflatoxin B_1 (^3H-AFB_1) with a specific activity of 15 Ci(555 GBq)/mmol was obtained from Moravek Biochemicals (Brea, CA, USA). The ^3H-AFB_1 was purified by LC using the system described below. Aflatoxin-B_1 (AFB_1), -B_{2a} (AFB_{2a}), -M_1 (AFM_1), -P_1 (AFP_1), -Q_1 (AFQ_1) and aflatoxicol (AFL), used as references in the LC, were purchased from SIGMA Chemical Co. (St Louis, MO, USA). Before use they were dissolved in methanol (25 ng/10 μl). Other chemicals were of analytical grade and obtained from regular commercial sources.

Tissues

The liver and the nasal olfactory mucosa were obtained from 5- to 7-year-old healthy cows (Swedish Red and White Breed) maintained on adequate diets with no history of previous exposure to AFB_1. The olfactory region of the nose was reached by sawing the cow's head transversally in two planes—one just in front of the eyes and one just behind the eyes—and then punching out the ethmoturbinates, covered with the olfactory epithelium, with a chisel (the olfactory epithelium is easily distinguishable by its brownish colour). The samples were taken immediately after slaughter and maintained at 0–4°C in 0.9% NaCl during the transport to the laboratory.

To prepare microsomes (for enzyme determinations), the liver and the nasal olfactory mucosa were homogenized in 0.02 M Tris–HCl buffer, pH 7.4, containing 1.15% KCl and centrifuged at 9000 *g* for 25 min. The supernatant was taken and centrifuged at 100 000 *g* for 1 h. The obtained microsomal pellet was resuspended in 0.1 M phosphate buffer, pH 7.0, to a protein concentration of 1.5 mg/ml. All procedures were performed at +4°C.

LC-conditions

The LC equipment consisted of a Constametric III pump (Laboratory Data Control/ LDC/ Milton Roy Co., Riviera Beach, FL, USA) connected to a Shimadzu spectrometric detector SPD-2A (Shimadzu Co., Analytical Instrument Plant, Kyoto, Japan) operated at 350 nm. The detector signal was processed by a Shimadzu CR 3A chromatopac. The column (4 × 300 mm) was packed with LiChrosorb RP-18, diameter 5 μm. The flow-rate was 0.8 ml/min. Two systems were used (A and B). In system A, the eluent was 30% acetonitrile buffered to pH 3.0 with phosphate buffer (μ = 0.1). (The composition of the buffer was: 15 ml 1 M H_3PO_4 and 100 ml 1 M NaH_2PO_4 diluted to 1000 ml by distilled H_2O). In this system AFB_{2a}, AFB_1 and AFL were eluted as well as separated peaks, whereas AFM_1, AFP_1 and AFQ_1 were eluted together. To separate the AFM_1, AFP_1 and AFQ_1, the peak from system A containing these metabolites was applied on system B, in which the eluent was 43% methanol buffered to pH 3.0 with the phosphate buffer.

In vitro microautoradiography

Pieces of nasal olfactory mucosa were incubated with 2 μCi (74 kBq, 0.13 nmol) of ^3H-AFB_1 in 2.5 ml 50 mM phosphate buffer, pH 7.4, containing 3 mM $MgCl_2$, 60 mM KCl, 0.4 mM NADP and 3 mM glucose-6-phosphate. The incubation was carried out during 1 h at 37°C under O_2-atmosphere in a shaking water-bath. After incubation the pieces were fixed in a buffered 4% formaldehyde-solution (pH 7.0). The tissues were dehydrated in an ethanol-series and embedded in Historesin™ (LKB-Produkter AB, Bromma, Sweden). Two-micrometre thick sections were cut on glass slides. Some sections were stained according to the periodic acid-Schiff (PAS) technique for the demonstration of mucosubstances. Other sections were stained by the Feulgen method for the demonstration of DNA. The stained sections were washed extensively in tap water and then covered in the dark with Kodak NTB-2 emulsion by dipping technique. Additional sections were dipped in the emulsion without previous staining. Exposure was carried out for a month at +4°C followed by photographic development and fixation. The sections which had been used for autoradiography without previous staining were post-stained with haematoxylin-eosin. The PAS-stained sections were post-stained with haematoxylin only, whereas no post-staining was performed with the Feulgen stained sections. It is assumed that the extensive extractions in various media during the fixation and embedding procedures will remove unbound metabolites and the autoradiograms therefore will show only cell-bound radioactivity.

Metabolism of AFB_1 in vitro

The *in vitro*-metabolism of AFB_1 was examined in the liver and the nasal olfactory mucosa. For the liver, samples weighing ~100 mg were sliced into small pieces by means of a pair of scissors. For the nasal olfactory mucosa, the mucosal layer was scraped off from the collagenous submucosa by a scalpel and these samples (~135 mg) were then used for the incubations. In the experiments, 0.3 μCi (11.1 kBq, 0.02 nmol) of ^3H-AFB_1 was incubated with the tissues for 1 h in 2.5 ml 50 mM phosphate buffer, pH 7.4, containing 3 mM $MgCl_2$, 60 mM KCl, 0.4 mM NADP and 3 mM glucose-6-phosphate. The incubations were terminated by adding 2.5 ml ice-cold methanol. The tissues were transferred in this medium to Potter–Elvehjem homogenizers and homogenized for ~1 min. Thereafter, 3 ml of chloroform were added. The mixture was shaken for 20 min and then centrifuged. The chloroform was removed and the procedure was repeated twice. The pH was then lowered to 3.0 by the addition of 300 μl of 1 M H_3PO_4-solution. The chloroform-extractions were then repeated several times, until no radioactivity could be detected in the organic phase (this was checked by liquid scintillation counting; see below). The partition properties of AFB_1 and the various AFB_1-metabolites used as references between chloroform and buffer at pH 7.4 and at pH 3.0 had been checked prior to the extractions. It was found that the procedure described above ensures total extraction of the AFB_1 and all the metabolites. After the final chloroform extraction the tissues were extracted repeatedly with 50 mM phosphate buffer, pH 7.4, again with shaking and centrifugations, until no more radioactivity could be removed. Separate pools were made of the combined chloroform and buffer extracts from each tissue. The remaining tissue pellets were dissolved in 10 ml 1 M NaOH. Aliquots of the extracts and the dissolved tissue pellets were taken for radioactivity determinations in a Packard Tri-Carb model 1900 CA liquid scintillation spectrometer using 10 ml of Hionic-fluor™ (Packard) (for the buffer extracts and the dissolved tissue-pellets) or 10 ml of Toluene scintillator™ (Packard) (for the chloroform extracts) as scintillation fluids. The protein contents of the dissolved tissue-pellets were determined according to Lowry *et al.* (23).

The chloroform extracts were used for LC. Thus, aliquots of the chloroform extracts were evaporated with N_2-gas and then redissolved in 300 μl 20% methanol containing 100 ng of each aflatoxin reference. One hundred microlitres of this solution was injected in LC system A to separate AFB_{2a}, AFB_1 and AFL. The absorbance at 350 nm was registered continuously and fractions were collected every 30 s for radioactivity determinations using 10 ml Hionic fluor as scintillation fluid. As indicated above, AFM_1, AFP_1 and AFQ_1 are eluted together in system

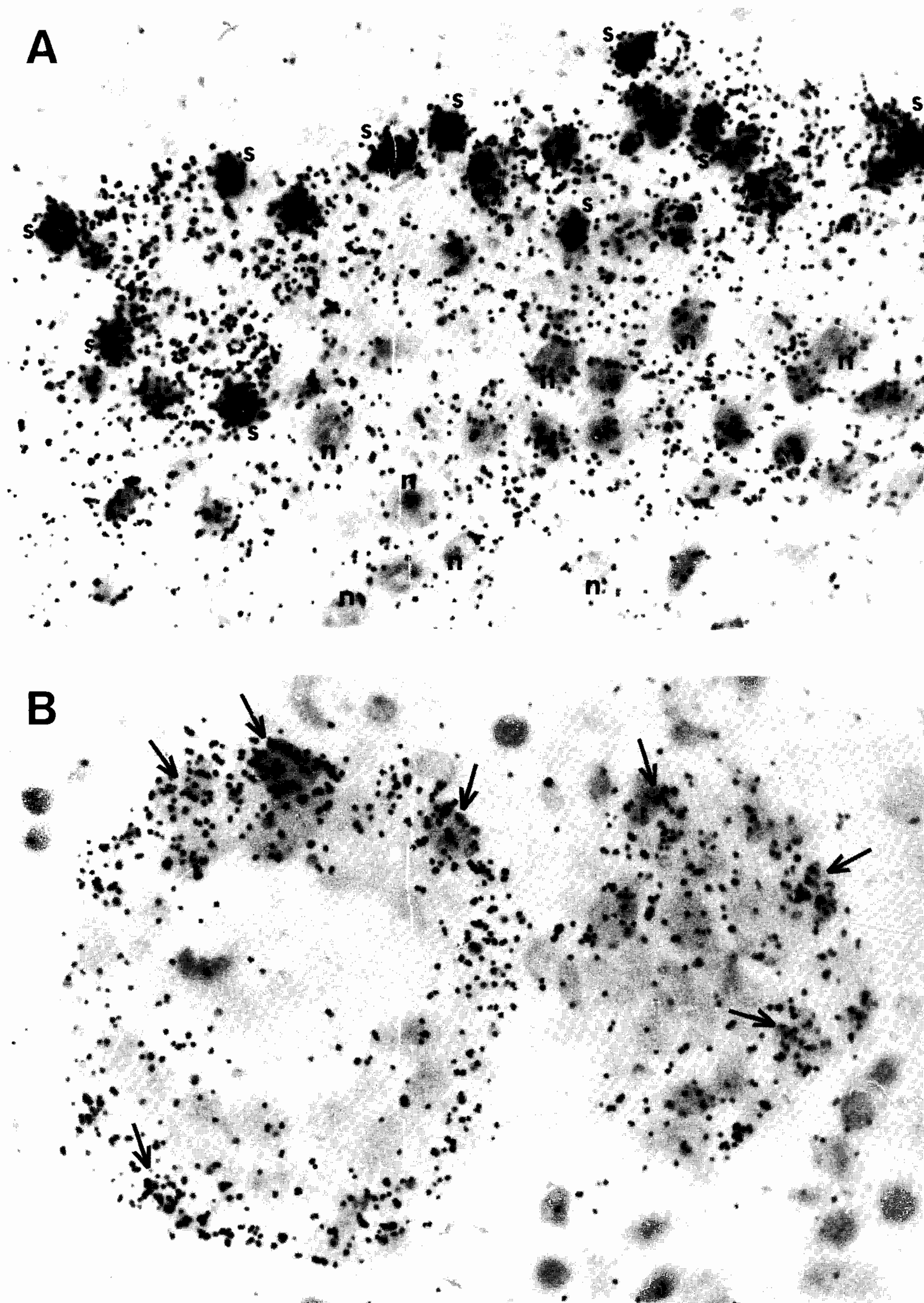

Fig. 1. Microautoradiograms of the nasal olfactory mucosa. (**A**) The surface epithelium with strongly labelled nuclei of sustentacular cells. The neuronal cell nuclei show a lower labelling. (**B**) The lamina propria mucosae with labelling of the cells of Bowman's glands. A few nuclei of these cells are also more strongly labelled than the cytoplasms. (s = sustentacular cell; n = neuronal cell; arrows show labelled nuclei of cells of Bowman's glands.) (PAS-hematoxylin; magnification × 1500.)

A, but are separated in system B. Thus, the peak containing these metabolites in system A was collected and extracted with chloroform. After evaporation, 100 μl 20% methanol was added and this was applied to system B. The analysis in system B was then performed in the same way as in system A.

Determination of cytochrome P-450, cytochrome b_5 and NADPH cytochrome c reductase activity

Cytochrome P-450 and cytochrome b_5 were determined according to Omura and Sato (24) and the NADPH cytochrome *c* reductase activity was determined according to Phillips *et al.* (25), in microsomal preparations of the nasal olfactory mucosa and the liver.

Statistical evaluation of data

Statistical significances were judged with the two-tailed Student's *t*-test of differences between mean values for independent samples.

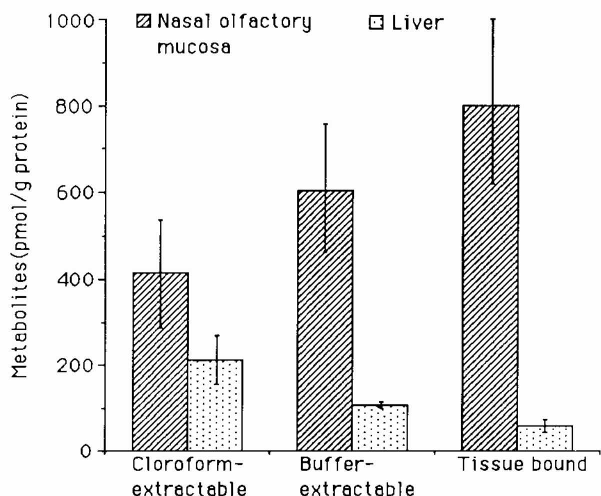

Fig. 2. Formation of chloroform-extractable, buffer-extractable and tissue bound metabolites after incubation of bovine nasal olfactory mucosa and liver with ^{3}H-AFB$_1$ (mean ± SD; $n = 9$). The values for the olfactory mucosa were significantly different from those of the liver ($P < 0.05$ for chloroform-extractable metabolites; $P < 0.001$ for buffer-extractable and tissue bound metabolites).

Results

Contents of cytochrome P-450 and cytochrome b_5 and NADPH cytochrome c reductase activity

The levels of cytochrome P-450 and cytochrome b_5 in the nasal olfactory mucosa were found to be respectively 26% and 37% of the levels observed in the liver. The NADPH cytochrome *c* reductase activity in the nasal olfactory mucosa was, however, five times higher than in the liver (Table I).

In vitro microautoradiography

The microautoradiography showed a distinct labelling of the sustentacular (supporting) cells in the apical portion of the olfactory surface epithelium (Figure 1A) (the sustentacular cell nuclei are easily recognized from the neuronal cell nuclei due to more elongated shape and a denser chromatin). The silver grains were much more numerous over the nuclei than over the cytoplasms of the sustentacular cells. Neuronal cells and basal cells in the mucosa were only weakly labelled. In the lamina propria mucosae there was a distinct labelling of Bowman's gland (Figure 1B). Some acini were strongly labelled whereas other showed a weaker radioactivity. In some cells of Bowman's glands the nuclei were more strongly labelled than the cytoplasms, but this phenomenon was not so apparent as in the sustentacular cells.

Metabolism of ^{3}H-AFB$_1$ in vitro

The nasal olfactory mucosa was found to have a high capacity to metabolize the ^{3}H-AFB$_1$. The highest levels of ^{3}H were bound to the tissue-macromolecules, but high amounts of metabolites were also found in the chloroform and buffer extracts (Figure 2). In the liver, the levels of metabolites were lower in all fractions in comparison with the nasal mucosa. The highest proportion of metabolites in the liver were recovered in the chloroform extracts.

Figure 3A and B shows LC-elution profiles from the chloroform extracts of the nasal olfactory mucosa. The LC-analyses showed that several metabolites were present both in

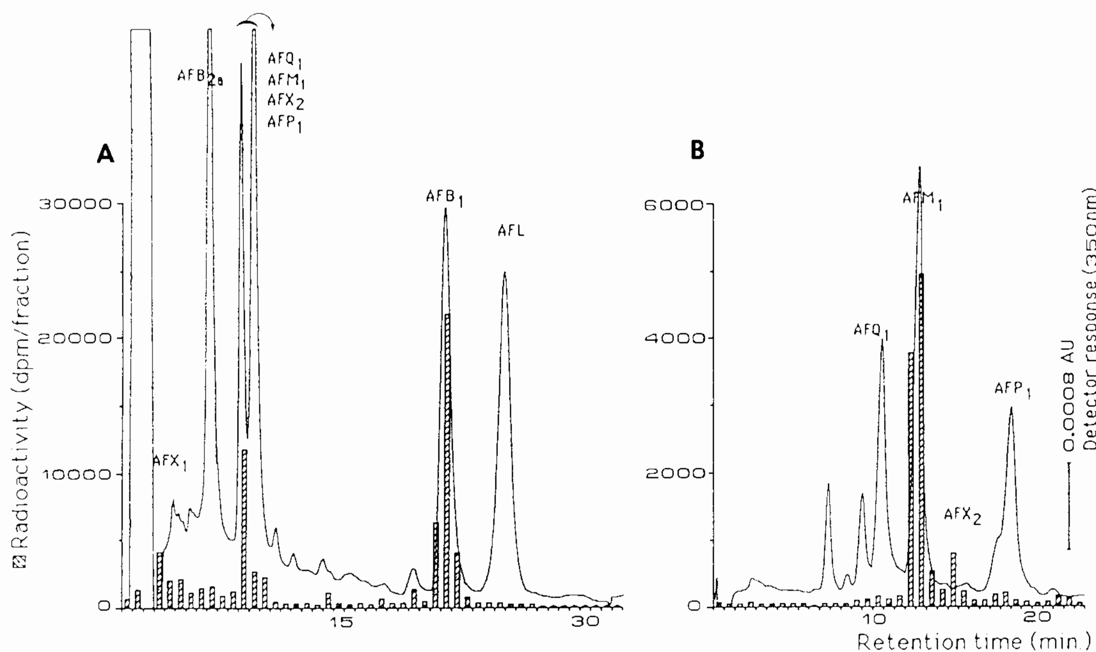

Fig. 3. LC-separation of AFB$_1$ and AFB$_1$-metabolites in the chloroform-extract of the nasal olfactory mucosa incubated with ^{3}H-AFB$_1$. (**A**) shows the chromatogram obtained with an eluent consisting of 30% acetonitril buffered to pH 3.0 with 0.1 M phosphate buffer. (**B**) shows the separation of the AFQ$_1$-, AFM$_1$-, AFX$_2$- and AFP$_1$-peaks when the peak in system (A) containing these metabolites is eluated with 43% methanol buffered to pH 3.0 with 0.1 M phosphate buffer.

Table II. Chloroform extractable metabolites formed upon incubation of ^{3}H-AFB_1 with bovine olfactory mucosa and liver (mean ± SD; n = 9)

Metabolite	Levels of metabolites (pmol/g protein)	
	Olfactory mucosa	Liver
AFX_1[a]	120 ± 51	Traces[c]
AFB_{2a}	ND[b]	23 ± 20
AFQ_1	Traces[c]	Traces[c]
AFM_1	258 ± 72	141 ± 63
AFX_2[a]	28 ± 5	14 ± 8
AFP_1	Traces[c]	8 ± 4
AFL	ND[b]	20 ± 7

[a]AFX_1 and AFX_2 are unidentified AFB_1-metabolites.
[b]ND = not detected.
[c]Low levels were found in some of the incubations.

the nasal olfactory mucosa and in the liver, although the proportions of the metabolites were different in the two tissues (Table II). The dominant peak in both tissues was AFM_1, but the level of this metabolite was about twice as high in the nasal olfactory mucosa as in the liver. AFL and AFB_{2a} were detected only in the extracts from the liver. Traces of AFQ_1 were detected in both tissues. AFP_1 was detected in the liver and in some extracts of the nasal mucosa. Two unidentified metabolites were eluted: one before the AFB_{2a}-peak (this metabolite is termed AFX_1) and one between the AFM_1- and AFP_1-peaks (this metabolite is termed AFX_2). The amount of AFX_1 was high in the nasal olfactory mucosa, but this metabolite was present only in traces in the liver. The amount of AFX_2 formed by the nasal olfactory mucosa was about twice as high as by the liver.

Discussion

The results of the present study show that the bovine nasal olfactory mucosa contains cytochrome P-450-enzymes and has a high capacity to metabolize AFB_1. The microautoradiography showed a distinct localization of tissue-bound metabolites in the sustentacular cells in the apical portion of the olfactory epithelium and in the cells of Bowman's glands. It has been shown by immunohistochemistry that the same structures of the nasal olfactory mucosa contain cytochrome P-450 and NADPH cytochrome P-450-reductase (26). It is likely that a bioactivation of AFB_1 occurs mainly in these cells. The microautoradiography showed a preferential labelling of the nuclei of the sustentacular cells, and the same phenomenon, although less marked, was seen also in the cells of Bowman's glands. It is possible that the microautoradiograms may reflect a specific affinity of the bioactivated AFB_1 for the DNA. It has been suggested that the AFB_1-epoxide-intermediate may interact with DNA in a non-covalent complex prior to the covalent reaction with the DNA (27). The AFB_1-epoxide is known to react more readily with double-stranded DNA than with single-stranded DNA (27).The N-7-guanin adduct appears to be the major adduct formed with the AFB_1-epoxide, but the guanines in some sequence-contexts appear to be more available for reaction than others (28). It has been shown that in the rat liver *in vivo* ~80% of the nuclear-bound AFB_1 is bound to the DNA (29).

The present observation that there was a higher metabolism of AFB_1 in the nasal olfactory mucosa than in the liver, in spite of a lower total content of cytochrome P-450 in the former than in the latter tissue, may be related to differences in the isoenzyme profile. Several compounds have been shown to be more actively metabolized in the nasal olfactory mucosa than in the liver in a number of species in spite of a lower total content of cytochrome P-450 in the nasal mucosa than in the liver (18–21). Multiple forms of cytochrome P-450 have been demonstrated in liver microsomes (30), but the isoenzyme profile of the olfactory cytochrome P-450 has not yet been characterized. Reed *et al.* (21) showed a higher NADPH cytochrome P-450 reductase: cytochrome P-450 ratio in the nasal mucosa than in the liver in rodents and proposed that this may facilitate microsomal electron transport and play an important role in the high drug-metabolizing activity of the nasal mucosa. The present study showed an even higher reductase: cytochrome P-450 ratio, suggesting that a similar mechanism may be involved in the potent metabolism of AFB_1 in the bovine nasal olfactory mucosa. In addition, the present study showed a higher cytochrome b_5: cytochrome P-450 ratio in the nasal mucosa than in the liver, and this may also promote the metabolism of the AFB_1. It has been shown that the metabolism of AFB_1 in hepatic microsomes can be enhanced by cytochrome b_5 (31).

The LC of the chloroform extracts showed that AFM_1 was the dominant lipid-soluble metabolite both in the liver and the nasal mucosa. However, in other aspects the metabolite pattern showed marked differences for the two tissues. This may again be related to differences in the P-450 isoenzyme-content in the two tissues. AFM_1 is known to be a major metabolite of AFB_1 in cattle *in vivo* (32). AFL is the only metabolite of AFB_1 produced by a soluble cytoplasmic reductase enzyme system. This metabolite was detected in the liver, but not in the nasal mucosa, indicating a lack of the reductase enzymes in the latter tissue. Two unidentified AFB_1-metabolites (designated AFX_1 and AFX_2 in Table II and Figure 2) were recovered in the chloroform extracts. In incubations of AFB_1 with rat or hamster liver microsomes, a major metabolite is 8,9-dihydro-8,9-dihydroxyaflatoxin B_1 (dihydrodiol) formed from the AFB_1-8,9-epoxide-intermediate (33). However, in the presence of DNA little or no formation of the dihydrodiol will occur (33) and it is therefore unlikely that AFX_1 or AFX_2 represent this metabolite.

Our study showed that higher levels of water-soluble AFB_1-metabolites were formed by the nasal olfactory mucosa than by the liver, but no attempts were made to identify these metabolites. It has been shown that AFB_1 may form sulphate-, glucuronide- and glutathione-conjugates in the liver (34–36). These water-soluble metabolites are probably formed also in the nasal mucosa, but further experiments have to be performed to elucidate this matter.

As mentioned previously, the nasal olfactory mucosa is a prevalent site of tumourigenesis in cattle in several developing countries (6–11), and it has been shown that affected animals sometimes suffer from severe aflatoxicosis (9). The histogenesis of the tumours has not always been conclusively established, but in several cases Bowman's glands have been considered to be the tissue which undergoes the initial malignant transformations (9,10). These glands showed a high localization of bound AFB_1-metabolites in our study. If AFB_1 plays a role in the etiology of the nasal tumours, the sustentacular cells, which showed strongly labelled nuclei in our study, might also be involved in the tumourigenesis.

In vitro metabolism of AFB_1 has been observed in tracheal explants of rabbits (37). Rats given AFB_1 intratracheally have been shown to develop tracheal tumours (38). Thus, a local exposure of a tissue with metabolizing capacity may promote carcinogenesis. It is possible that inhalation of AFB_1-contaminated grain dust particles by cattle, leading to a

local exposure of the olfactory mucosa, may increase the risk for tumour induction in this tissue.

Acknowledgements

This study was supported by a grant from the Swedish Council for Forestry and Agricultural Research.

References

1. Busby,W.F.,Jr and Wogan,G.N. (1984) Aflatoxins. In Searle,C.E. (ed.), *Chemical Carcinogens*, ACS Monograph 182, American Chemical Society, Washington, Vol. 2, pp. 945–1136.
2. Patterson,D.S.P. (1983) Aflatoxicosis in farm animals. *Vet. Res. Commun.*, **7**, 135–140.
3. Vaid,J., Dawra,R.K., Sharma,O.P. and Negi,S.S. (1981) Chronic aflatoxicosis in cattle. *Vet. Hum. Toxicol.*, **23**, 436–438.
4. Brucato,M., Sundlof,S.F., Bell,J.U. and Edds,G.T. (1986) Aflatoxin B_1 toxicosis in dairy calves pretreated with selenium-vitamin E. *Am. J. Vet. Res.*, **47**, 179–183.
5. Peers,F.G. and Linsell,C.A. (1977) Dietary aflatoxins and human primary liver cancer. *Ann. Nutr. Aliment.*, **31**, 1005–1018.
6. Amaral,L.B.S. and Nesti,A. (1963) Incidência de cancer em bovinos e suínos. *Biologico*, **29**, 30–31.
7. Nazario,W., Valente,F.A.T., Portugal,M.A.S.C., Amaral,L.B.S. and Nesti,A. (1966) Carcinomas in the ethmoidal sinus of bovines and swines. *Proceedings of the 5th Panamerican Congress on Veterinary Medicine and Zootechnics*, Caracas, Vol. 2, 832–833.
8. Inada,T. and Tokarnia,C.H. (1973) Histopathological and histochemical studies of two cases of enzootic ethmoidal tumour in cattle. *Pesq. Agropec. Bras., Ser. Vet.*, **8**, 85–88.
9. Pospischil,A., Haenichen,T. and Schaeffler,H. (1979) Histological and electron microscopic studies on endemic ethmoidal carcinomas in cattle. *Vet. Pathol.*, **16**, 180–190.
10. Sreekumaran,T. and Rajan,A. (1983) Histology and histochemistry of endemic ethmoid carcinoma in bovines. *Indian J. Cancer*, **20**, 10–14.
11. Sreekumaran,T. and Rajan,A. (1983) Epidemiology of ethmoid carcinoma in bovines. *Indian J. Cancer*, **20**, 5–9.
12. Sorenson,W.G., Simpson,J.P., Peach,M.J.,III, Thedell,T.D. and Olenchock,S.A. (1981) Aflatoxin in respirable corn dust particles. *J. Toxicol. Environ. Health*, **5**, 669–672.
13. Burg,W.R. and Shotwell,O.L. (1984) Aflatoxin levels in air-borne dust generated from contaminated corn during harvest and at an elevator in 1980. *J. Assoc. Off. Anal. Chem.*, **67**, 309–312.
14. Lewis,G., Markson,L.M. and Allcroft,R. (1967) The effect of feeding toxic groundnut meal to sheep over a period of five years. *Vet. Rec.*, **80**, 312–314.
15. Adamson,R.H. and Sieber,S.M. (1979) The use of nonhuman primates for chemical carcinogenesis studies. In Coulston,F. (ed.), *Regulatory Aspects of Carcinogenesis and Food Additives: The Delaney Clause*, Academic Press, New York, Vol. 2, pp. 275–302.
16. Goerttler,K., Löhrke,H., Schweizer,H.-J. and Hesse,B. (1980) Effects of aflatoxin B_1 on pregnant inbred Sprague–Dawley rats and their F_1 generation. A contribution to transplacental carcinogenesis. *J. Natl. Cancer Inst.*, **64**, 1349–1354.
17. Hayes,R.B., van Nieuwenhuize,J.P., Raatgever,J.W. and ten Kate,F.J.W. (1984) Aflatoxin exposures in the industrial setting: an epidemiological study of mortality. *Food Chem. Toxic.*, **22**, 39–43.
18. Dahl,A.R., Hadley,W.M., Hahn,F.F., Benson,J.M and McClellan,R.O. (1982) Cytochrome P-450-dependent monooxygenases in the olfactory epithelium of dogs: possible role in tumorigenicity. *Science*, **216**, 57–59.
19. Tjälve,H. and Löfberg,B. (1983) Extrahepatic sites of metabolism of carbon tetrachloride in rats. *Chem.-Biol. Interact.*, **46**, 299–316.
20. Brittebo,E.B. and Tjälve,H. (1983) Metabolism of *N*-nitrosamines by the nasal mucosa. In Reznik,G. and Stinson,S.F. (eds), *Nasal Tumors in Animals and Man*, CRC Press, Inc., Boca Raton, Florida, Vol. III, pp. 233–250.
21. Reed,C.J., Lock,E.A. and De Matteis,F. (1986) NADPH: cytochrome P-450 reductase in olfactory epithelium. Relevance to cytochrome P-450-dependent reactions. *Biochem. J.*, **240**, 585–592.
22. Mannering,G.J., Kuwahara,S. and Omura,T. (1974) Immunochemical evidence for the participation of cytochrome b_5 in the NADH synergism of the NADPH-dependent mono-oxidase system of hepatic microsomes. *Biochem. Biophys. Res. Commun.*, **57**, 476–481.
23. Lowry,O.H., Rosebrough,N.J., Farr,A.L. and Randall,R.J. (1951) Protein Measurement with the folin phenol reagent. *J. Biol. Chem.*, **193**, 265–275.
24. Omura,T. and Sato,R. (1964) The carbon monoxide-binding pigment of liver microsomes. *J. Biol. Chem.*, **239**, 2370–2378.
25. Phillips,A.H. and Langdon,R.G. (1962) Hepatic triphosphopyridine nucleotide-cytochrome *c* reductase: isolation, characterization, and kinetic studies. *J. Biol. Chem.*, **237**, 2652–2660.
26. Voigt,J.M., Guengerich,F.P. and Baron,J. (1985) Localization of a cytochrome P-450 isozyme (cytochrome P-450 PB-B) and NADPH-cytochrome P-450 reductase in rat nasal mucosa. *Cancer Lett.*, **27**, 241–247.
27. Misra,R.P., Muench,K.F. and Humayun,M.Z. (1983) Covalent and noncovalent interactions of aflatoxin with defined deoxyribonucleic acid sequences. *Biochemistry*, **22**, 3351–3359.
28. Benasutti,M., Ejadi,S., Whitlow,M.C. and Loechler,E.L. (1988) Mapping the binding site of aflatoxin B_1 in DNA: systematic analysis of the reactivity of aflatoxin B_1 with guanines in different DNA sequences. *Biochemistry*, **27**, 472–481.
29. Groopman,J.D., Busby,W.F.,Jr and Wogan,G.N. (1980) Nuclear distribution of aflatoxin B_1 and its interaction with histones in rat liver *in vivo*. *Cancer Res.*, **40**, 4343–4351.
30. Lu,A.Y.H. and West,S.B. (1980) Multiplicity of mammalian microsomal cytochromes P-450. *Pharmacol. Rev.*, **31**, 277–295.
31. Ueno,Y., Ishii,K., Omata,Y., Kamataki,T. and Kato,R. (1983) Specificity of hepatic cytochrome P-450 isoenzymes from PCB-treated rats and participation of cytochrome b_5 in the activation of aflatoxin B_1. *Carcinogenesis*, **4**, 1071–1073.
32. Subblefield,R.D., Pier,A.C., Richard,J.L. and Shotwell,O.L. (1983) Fate of aflatoxins in tissues, fluids, and excrements from cows dosed orally with aflatoxin B_1. *Am. J. Vet. Res.*, **44**, 1750–1752.
33. Lin,J.-K., Kennan,K.A., Miller,E.C. and Miller,J.A. (1978) Reduced nicotinamide adenine dinucleotide phosphate-dependent formation of 2,3-dihydro-2,3-dihydroxyaflatoxin B_1 from aflatoxin B_1 by hepatic microsomes. *Cancer Res.*, **38**, 2424–2428.
34. Wei,C.I., Marshall,M.R. and Hsieh,D.P.H. (1985) Characterization of water-soluble glucuronide and sulphate conjugates of aflatoxin B_1. 1. Urinary excretion in monkey, rat and mouse. *Food Chem. Toxic.*, **23**, 809–819.
35. Wei,C.I. and Hsieh,D.P.H. (1985) Characterization of water-soluble glucuronide and sulphate conjugates of aflatoxin B_1. 2. Studies in primary cultures of rat hepatocytes. *Food Chem. Toxic.*, **23**, 821–825.
36. Degen,G.H. and Neumann,H.-G. (1978) The major metabolite of aflatoxin B_1 in the rat is a glutathione conjugate. *Chem.-Biol. Interact.*, **22**, 239–255.
37. Coulombe,R.A.,Jr, Wilson,D.W., Hsieh,D.P.H., Plopper,C.G. and Serabjit-Singh,C.J. (1986) Metabolism of aflatoxin B_1 in the upper airways of the rabbit: role of the nonciliated tracheal epithelial cell. *Cancer Res.*, **46**, 4091–4096.
38. Dickens,F., Jones,H.E.H. and Waynforth,H.B. (1966) Oral, subcutaneous, and intratracheal administration of carcinogenic lactones and related substances: the intratracheal administration of cigarette tar in the rat. *Br. J. Cancer*, **20**, 134–144.

Received on December 22, 1988; revised on March 15, 1989; accepted on March 20, 1989