

## Bioactivation of aflatoxin B<sub>1</sub> in the bovine olfactory mucosa: DNA binding, mutagenicity and induction of sister chromatid exchanges

Hans Tjälve<sup>1</sup>, Pia Larsson<sup>1</sup>, Christer Andersson<sup>2,3</sup> and Leif Busk<sup>3,4</sup>

<sup>1</sup>Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, The Swedish University of Agricultural Sciences, Box 573, S-751 23 Uppsala. <sup>2</sup>Department of Genetics, University of Uppsala, Box 7003, S-750 07 Uppsala. <sup>3</sup>Department of Toxicology, Swedish National Food Administration, Box 622, S-751 26 Uppsala and <sup>4</sup>Department of Pharmacology, Medical Products Agency, Box 26, S-751 03 Uppsala, Sweden

**Nasal olfactory tumours occur in cattle in relatively high frequencies in several developing countries. Since affected animals sometimes show signs of severe aflatoxicosis, a role of aflatoxin B<sub>1</sub> (AFB<sub>1</sub>) in tumorigenesis can be proposed. The results of the present study show that microsomal preparations of the bovine olfactory mucosa have a much higher ability than liver microsomes to induce covalent binding of AFB<sub>1</sub> to calf thymus DNA and to microsomal proteins. The major DNA adduct formed was 8,9-dihydro-8-(N<sup>7</sup>-guanyl)-9-hydroxy-aflatoxin B<sub>1</sub>. Incubations of microsomal preparations of the bovine nasal olfactory mucosa with glutathione (GSH) and cytosolic fractions of the nasal mucosa resulted in decreased AFB<sub>1</sub> DNA binding. A more pronounced decrease was observed when cytosolic fractions of mouse liver were added to the incubations. Mouse liver is known to contain a glutathione-S-transferase with a high ability to scavenge the reactive AFB<sub>1</sub>-epoxide via conjugation to GSH. Our results indicate that AFB<sub>1</sub>-GSH conjugation occurs less efficiently in the bovine nasal olfactory mucosa than in the mouse liver. Supernatant preparations (9000 g) of the bovine nasal olfactory mucosa incubated with AFB<sub>1</sub> were shown to have the capacity to induce a strong genotoxic response both as regards induction of gene mutations in *Salmonella typhimurium* TA100 and the induction of sister chromatid exchanges in Chinese hamster ovary cells. Preparations of the bovine liver (9000 g) has a much lower ability to induce these effects. The results of the present study show that the bovine nasal olfactory mucosa has a high AFB<sub>1</sub>-bioactivating capacity, which can be related to the potent DNA damaging and mutagenic effects observed. It is considered that our results support the assumption that AFB<sub>1</sub> plays a role in the aetiology of nasal tumours in cattle.**

### Introduction

The aflatoxins are extremely potent mycotoxins produced by strains of the fungi *Aspergillus flavus* and *Aspergillus parasiticus* (1). Farm animals are exposed orally to the aflatoxins primarily

\*Abbreviations: AFB<sub>1</sub>, aflatoxin B<sub>1</sub>; GSH, glutathione; GST, glutathione-S-transferase; AFB<sub>1</sub>-N<sup>7</sup>-Gua, 8,9-dihydro-8-(N<sup>7</sup>-guanyl)-9-hydroxy-aflatoxin B<sub>1</sub>; SCE, sister chromatid exchange; CHO cells, Chinese hamster ovary cells; BSA, bovine serum albumin; CPBA, 3-chloroperoxybenzoic acid; LC, liquid chromatography; DMSO, dimethylsulfoxide; BrdUrd, 5-bromodeoxyuridine; AFB<sub>1</sub>-diol, 8,9-dihydro-8,9-dihydroxy-AFB<sub>1</sub>.

via feed concentrates (2). In addition, exposure may take place via inhalation, since significant amounts of these mycotoxins can be present in respirable particles of grain dust (3,4). The growth of the fungi is favoured by relatively high temperature and humidity and contamination with aflatoxins is therefore most common in tropical and subtropical areas.

We have recently shown that the bovine nasal olfactory mucosa has a high capacity to metabolize aflatoxin B<sub>1</sub> (AFB<sub>1</sub>\*) to lipid-soluble, water-soluble and tissue-bound metabolites (5). This study was performed to evaluate the assumption that AFB<sub>1</sub> may play a role in the aetiology of nasal tumours in cattle. Such tumours, which develop from the olfactory mucosa in the ethmoidal region of the nose, are observed in cattle in severe developing countries (6–8). In some areas of India and middle America the tumour incidence has been reported to reach endemic proportions (6,7). The aetiology of the tumours has not been determined. It has been proposed that a virus may be the causative agent (7,8). Affected animals sometimes show signs of several aflatoxicosis suggesting that aflatoxins may be involved (6,8).

AFB<sub>1</sub> is inactive *per se* and must be metabolized to the AFB<sub>1</sub>-8,9-epoxide in order to bind to cellular macromolecules, such as DNA and proteins (9,10). The activation appears to be carried out mainly by the cytochrome P450 system (1). The epoxide may undergo further detoxification, primarily via conjugation with glutathione (GSH) by the action of glutathione-S-transferase (GST) (11,12).

The major AFB<sub>1</sub>-DNA adduct formed has been shown to be 8,9-dihydro-8-(N<sup>7</sup>-guanyl)-9-hydroxy-aflatoxin B<sub>1</sub> (AFB<sub>1</sub>-N<sup>7</sup>-Gua) (13,14). The DNA binding and subsequent genetic effects are critical for cell transformation and tumorigenesis.

In the present study, we have investigated the ability of microsomal preparations from the bovine olfactory mucosa to induce AFB<sub>1</sub> binding to calf thymus DNA and to microsomal proteins. Comparative experiments were performed with bovine liver microsomes. In addition, we examined the influence of GSH, and cytosolic fractions from bovine nasal olfactory mucosa and liver and cytosolic fractions from mouse liver on the level of AFB<sub>1</sub>-DNA binding mediated by microsomal preparations of bovine nasal olfactory mucosa. The aim of this experiment was to estimate the activity of GST to scavenge the AFB<sub>1</sub>-8,9-epoxide. It is known that mouse liver contains a GST-isoenzyme, which exhibits high activity towards the AFB<sub>1</sub>-8,9-epoxide (15) and the inclusion of mouse liver cytosols in our experiments was done to obtain comparative data with the bovine nasal mucosa and liver. We also examined the ability of 9000 g supernatant preparations of the bovine nasal olfactory mucosa and liver to promote induction of mutations in *Salmonella typhimurium* TA100 and to induce sister chromatid exchanges (SCE) in Chinese hamster ovary (CHO) cells in the presence of AFB<sub>1</sub>. These experiments aimed to determine the mutagenic and DNA damaging effect of AFB<sub>1</sub> bioactivation in the bovine nasal olfactory mucosa and liver. Parts of the data on the DNA binding of AFB<sub>1</sub> have been previously reported at a conference (16).

## Materials and methods

### Chemicals

[G-<sup>3</sup>H]Aflatoxin B<sub>1</sub> ([<sup>3</sup>H]AFB<sub>1</sub>) with a sp. act. of 21 Ci (777 GBq)/mmol was obtained from Moravak Biochemicals (Brea, CA, USA). AFB<sub>1</sub>, calf thymus DNA, bovine serum albumin (BSA), reduced glutathione (GSH) and glucose-6-phosphate dehydrogenase were purchased from Sigma Chemical Co. (St Louis, MO, USA). 3-Chloroperoxybenzoic acid (CPBA) was purchased from Fluka Chemic AG (Buchs, Switzerland). Other chemicals were of analytical grade and obtained from regular commercial sources.

### Tissues

Nasal olfactory mucosa and liver were obtained from 5–7 year old healthy cows (Swedish Red and White Breed) maintained on adequate diets and with no history of previous exposure of AFB<sub>1</sub>. The olfactory region of the nose was removed as described (5). The tissues were taken immediately after death from cows slaughtered at a local abattoir. Mouse livers were taken from female C57Bl-mice. The mice used for this purpose were obtained from A-lab (Uppsala, Sweden) and were given a standard pellet diet (Ewos AB, Södertälje, Sweden) and tap water *ad libitum*.

### Preparation of subcellular fractions

For preparation of 9000 *g* supernatants (designated as the S-9 fraction), postmicrosomal supernatants (designated as the cytosolic fraction), microsomes of bovine nasal olfactory mucosa and liver, and cytosolic fractions of mouse liver, the respective tissue was homogenized in 50 mM Tris-HCl buffer pH 7.5, containing 50 mM KCl, and then centrifuged at 9000 *g* for 20 min. The supernatants (the S-9 fraction) obtained from four cows were individually stored at -70°C to be used in the *Salmonella* mutagenicity test and the SCE assay. For the other experiments S-9 fractions from six additional cows were pooled and centrifuged at 100000 *g* for 1 h. The resulting supernatants (the cytosolic fraction) were collected and stored at -70°C. The microsomes were resuspended in the Tris-HCl buffer and centrifuged at 100 000 *g* for 1 h. The microsomal pellets were again resuspended in the Tris-HCl buffer and stored at -70°C until used. To obtain the mouse liver cytosolic fractions, tissues from eight animals were pooled.

### Preparation of AFB<sub>1</sub>-N<sup>7</sup>-Gua

AFB<sub>1</sub>-modified DNA was prepared with CPBA as the oxidizing agent mainly as described by Martin and Garner (17). In the procedure, [<sup>3</sup>H] AFB<sub>1</sub> (20 μCi, 0.3 mg), 5 mg calf thymus DNA and 1.4 mg CPBA were incubated overnight under stirring in a two-phase system consisting of 50 mM phosphate buffer pH 6.0 and dichloromethane (1:1). After the incubation, the aqueous phase was extracted three times with chloroform:isoamylalcohol (24:1) before the DNA was precipitated with three volumes of ice cold ethanol and redissolved in 3 ml 15 mM NaCl/1.5 mM Na-citrate buffer, pH 6.5. Aliquots were analysed in a Packard Tri-Carb Model 1900 CA liquid scintillation spectrometer. The DNA content was determined according to the method of Burton (18) as modified by Giles and Myers (19).

The AFB<sub>1</sub>-modified DNA was hydrolysed and the hydrolysates were cleaned on a C<sub>18</sub>Sep-Pak column (Waters Associates) by procedures described by Lin *et al.* (14) and Groopman *et al.* (20). The AFB<sub>1</sub>-N<sup>7</sup>-Gua was isolated on liquid chromatography (LC) using the system described below. The absorbance spectra of the isolated AFB<sub>1</sub>-N<sup>7</sup>-Gua were taken in 0.1 M HCl and 0.1 M NaOH, as described by Essigmann *et al.* (13). The absorbance maxima were found to be 238, 263 and 269 nm, which correlates with the data of Essigmann *et al.* (13).

One compound eluted in the LC system prior to AFB<sub>1</sub>-N<sup>7</sup>-Gua. This is probably 8,9-dihydro-8,9-dihydroxy-AFB<sub>1</sub> (AFB<sub>1</sub>-diol). It was found that the spectral characteristics of this compound in acidic (λ<sub>max</sub> 360, 265, 220) and basic (λ<sub>max</sub> 400, 288, 246) solutions and the LC retention time coincided with data reported for the AFB<sub>1</sub>-diol by Croy and Wogan (21). The AFB<sub>1</sub>-diol may be formed by hydrolytic cleavage of the AFB<sub>1</sub> adducted to the DNA (21).

### LC conditions

The LC equipment consisted of a Beckman System Gold Programmable Solvent Module 126 (Beckman Instrument Inc., Alex Div., CA, USA) connected to an LDC Spectromonitor III (Milton Ray Co., FL, USA) working at 365 nm. The column was a 30 cm long μBondapak C<sub>18</sub> (Waters, Millipore, MA, USA) eluted with a flowrate of 1 ml/min and a linear ethanol/20 mM K-acetate (pH 5) gradient from 10 to 18% ethanol over 25 min (0–5 min 10% ethanol; 5–30 min 10–18% ethanol). Radioactivity was measured by taking fractions every 30 s.

### Formation of DNA- and protein-bound AFB<sub>1</sub> metabolites by microsomes from bovine nasal olfactory mucosa and liver

The incubations were carried out in a total volume of 2 ml in 50 mM Tris-HCl pH 7.5, containing 0.8 mM NADP, 5 mM glucose-6-phosphate, 0.4 U glucose-6-phosphate dehydrogenase/ml, 3 mM MgCl<sub>2</sub>, 50 mM KCl, 1 mg calf thymus DNA and 0.2 μCi [<sup>3</sup>H]AFB<sub>1</sub> (0.2–2 μM AFB<sub>1</sub>).

The incubations were initiated by adding bovine olfactory mucosa or liver microsomes in amounts corresponding to 1.0 mg protein per incubation. The incubations were carried out for 15 min in an O<sub>2</sub> atmosphere at 37°C. The metabolic reactions were stopped by adding 0.5 ml 4.5 M NaCl and 0.1 ml 3% SDS. The samples were extracted twice with 2 ml chloroform:isoamylalcohol (90:2). The DNA was precipitated from the aqueous layer by adding 3 volumes of ice cold ethanol and the amount of DNA was determined as described above. The recovery of the DNA was ~90%. Aliquots were taken for scintillation counting. The level of AFB<sub>1</sub> modification of calf thymus DNA was determined by assuming an average weight of 326 per nucleotide residue. The DNA was hydrolysed and analysed by LC as described above. The protein pellets were further extracted with 1% SDS and acetone, using the method of Baker and Van Dyke (22). The extracted protein pellet was dissolved in 1 M NaOH, aliquots were taken for radioactivity counting and protein determinations according to Lowry *et al.* (23). Incubations which were performed in the presence of the reducing agent dithionite served as controls. In the control incubations there was no AFB<sub>1</sub> binding either to protein or DNA.

### Effects of GSH and cytosolic fractions on the binding of bioactivated AFB<sub>1</sub> to DNA

The binding of bioactivated AFB<sub>1</sub> to DNA in the presence of GSH and cytosolic fractions was examined using an approach similar to those described by Monroe and Eaton (24) and Quinn *et al.* (25). In these incubations, GSH (5 mM) and cytosolic fractions of mouse liver, bovine liver or bovine olfactory mucosa (in concentrations corresponding to 0.75 or 1.5 mg protein/2 ml incubation solution) were added. Bioactivation was achieved by microsomes from bovine olfactory mucosa (0.25 mg of microsomal protein/2 ml incubation solution). The incubation procedure, extractions and analyses were performed as described above. Incubations in which GSH was excluded and the cytosolic fractions were substituted with 0.75 or 1.5 mg BSA/incubation served as controls.

### *Salmonella* mutagenicity test

Reversion of *S. typhimurium* his<sup>-</sup> was used to assay mutagenicity. *S. typhimurium* strain TA100 was obtained from Dr B.N. Ames (University of California, Berkeley). The mutagenicity tests, including the preparation of S-9 mix, were performed as described by Maron and Ames (26). The amount of cofactors was the same regardless of the protein concentration. The bacteria were grown in nutrient broth overnight. To a 2 ml aliquot of 45°C warm top agar (containing 0.5% agar, 0.5% NaCl, 200 μM histidine and 200 μM biotin) was added AFB<sub>1</sub> dissolved in 100 μl dimethylsulfoxide (DMSO) or DMSO only (controls), 0.5 ml of S-9 mix prepared from bovine olfactory mucosa or liver and 0.1 ml of the bacterial suspension. The mixture was poured onto a Petri dish containing 20 ml Vogel-Bonner agar. After incubation at 37°C for 48 h colonies with a diameter >0.2 mm were counted in a Bio Tron II Automated Colony Counter. The bovine S-9 fractions were obtained from nasal olfactory mucosa and liver from four cows. Since the effect obtained in the presence of the metabolizing systems from the four animals were very similar, the data were pooled.

### Assay of SCE

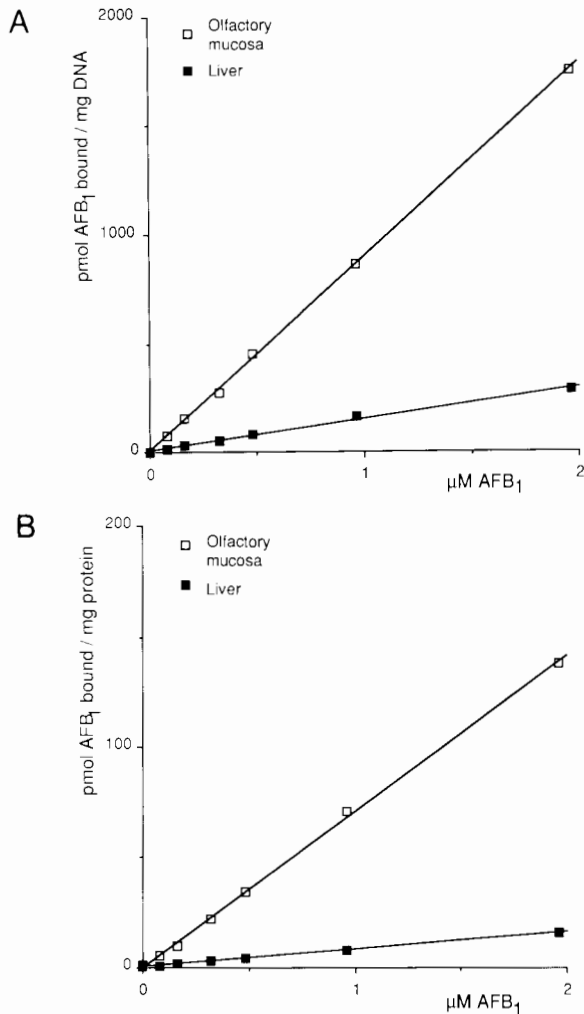
CHO cells of the subline K<sub>1</sub> were cultured in Ham's F-10 supplemented with 15% newborn calf serum, 1.0 mM L-glutamine, 100 IU/ml penicillin and 100 μg/ml streptomycin. The cultures were grown in 5% CO<sub>2</sub> atmosphere at 37°C and subcultured the day before starting an experiment. All media components were from Flow Laboratories.

The experiments were initiated by adding 5-bromodeoxyuridine (BrdUrd) to the cell cultures at a final concentration of 10 μM. After 12 h (i.e. approximately one cell cycle) the medium was discharged. The cells were then exposed in F-10 for 1 h to AFB<sub>1</sub> in the absence or presence of 10% of S-9 mix (by volume), the metabolizing system being obtained from bovine olfactory mucosa or liver. The composition of the S-9 mix was the same as in the *Salmonella* mutagenicity test (26). AFB<sub>1</sub> was added to the cells dissolved in DMSO. The concentration of the DMSO during the treatments usually did not exceed 1% and was never >3%. The DMSO concentrations did not influence the frequency of SCE (data not shown). After the end of the 1 h AFB<sub>1</sub> exposure, the cells were washed once with F-10, supplied with fresh medium containing 10 μM BrdUrd and grown for another 13 h before being harvested. To collect metaphases the cells were exposed to 2.5 μM colchicine during the last 2 h of culture. With the exception of the manipulations with the cultures, which were performed under yellow safety light, the entire experiment was performed in the dark. After fixation in 3:1 methanol:glacial acetic acid, air-dried preparations were made according to a standard protocol and stained for SCE analysis as described by Perry and Wolff (27). For each treatment at least 25 cells were scored for the frequency of SCE.

As for the *Salmonella* mutagenicity test, the data from the four cows used were very similar and were therefore pooled.

### Statistical analysis

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



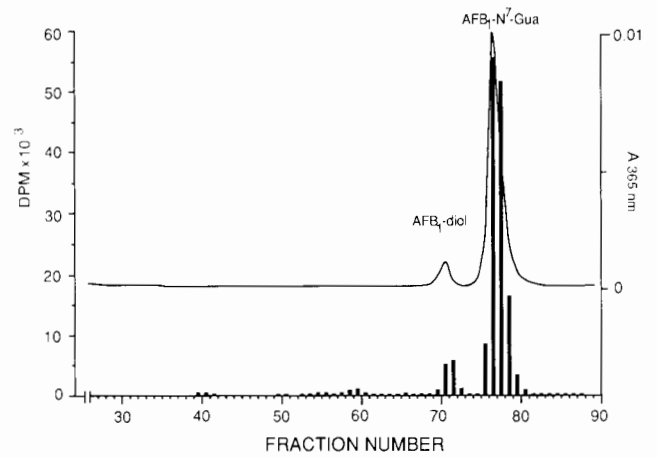
**Fig. 1.** Binding of microsome-activated AFB<sub>1</sub> to DNA (A) and protein (B). One milligram of DNA and 1 mg of bovine olfactory or liver microsomal protein were present in the incubation media (mean of duplicate experiments; the individual values differed by <2% from the means).

## Results

### Formation of DNA- and protein-bound AFB<sub>1</sub> metabolites by microsomes from bovine olfactory mucosa and liver

The binding of [<sup>3</sup>H]AFB<sub>1</sub> to DNA and to microsomal proteins was found to be much higher in incubations with olfactory microsomes than liver microsomes. The degree of binding was linearly related to the [<sup>3</sup>H]AFB<sub>1</sub> concentrations examined (Figure 1A and B). Both in the nose and the liver the microsome-activated AFB<sub>1</sub> binding to the DNA was much higher than to the microsomal proteins. It was calculated that at the highest AFB<sub>1</sub> concentration used (2 μM) one AFB<sub>1</sub> molecule was bound/1700 nucleotides in incubations with the olfactory microsomes, whereas one AFB<sub>1</sub> molecule was bound/10000 nucleotides in incubations with the liver microsomes.

The LC profiles in Figure 2 show the radioactivity distribution of DNA-hydrolysates, from incubations with bovine olfactory microsomes. The peaks in Figure 2 have the same retention times as the references (AFB<sub>1</sub>-N<sup>7</sup>-Gua and AFB<sub>1</sub>-diol). The major radioactivity peak coelutes with the AFB<sub>1</sub>-N<sup>7</sup>-Gua and the minor radioactivity peak coelutes with the AFB<sub>1</sub>-diol. About 80% of the radioactivity in the extracted and isolated DNA is recovered as AFB<sub>1</sub>-N<sup>7</sup>-Gua after hydrolysis.



**Fig. 2.** LC separation of the hydrolysis products of AFB<sub>1</sub>-modified DNA obtained from incubations with 1 mg bovine olfactory microsomes, 1 mg DNA and 1.92 μM AFB<sub>1</sub> (0.9 μCi [<sup>3</sup>H]AFB<sub>1</sub>). Absorbance at 365 nm (—) and amount of <sup>3</sup>H (■) in fractions taken every 30 s are shown.

**Table I.** Effect of GSH (5 mM) and addition of cytosolic fractions on bovine olfactory microsome mediated AFB<sub>1</sub> binding to DNA<sup>a</sup>

Conditions	DNA-bound AFB <sub>1</sub> (%)	
	Amount of BSA or cytosolic protein added (mg)	
	0.75	1.50
BSA without GSH	100	100
BSA with GSH	95	95
Mouse liver cytosol	70	40
Bovine olfactory cytosol	90	50
Bovine liver cytosol	100	65

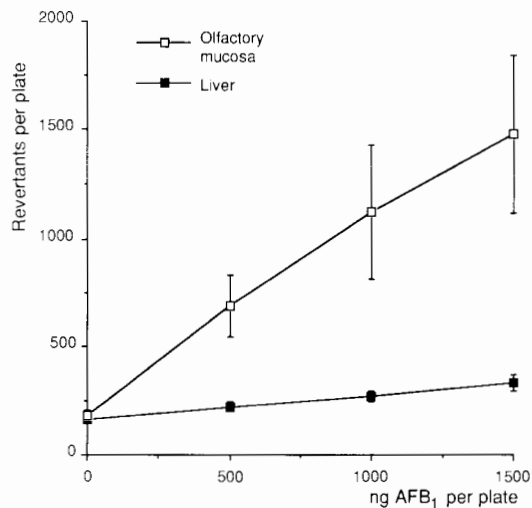
<sup>a</sup>The incubations were performed in triplicate; the deviation from the mean values were <6%. For incubation conditions see Materials and methods.

### Effect of GSH and cytosolic fractions on the binding of bioactivated AFB<sub>1</sub> to DNA

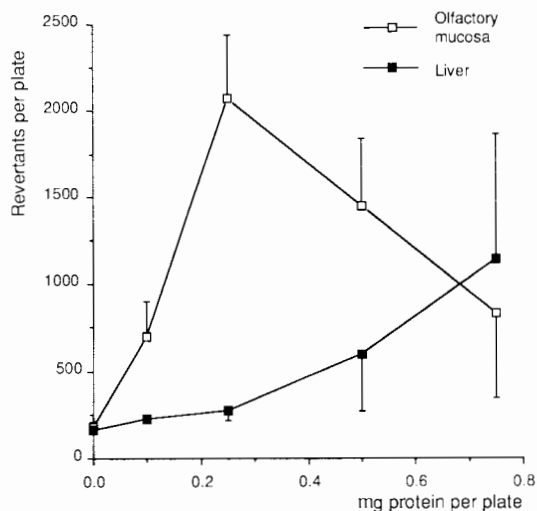
The AFB<sub>1</sub> binding to DNA was decreased in the presence of GSH and cytosolic fractions (Table I). The inhibition of the binding of the bioactivated AFB<sub>1</sub> was higher in the incubations performed with 1.5 mg of cytosolic protein as compared with 0.75 mg of cytosolic protein. The mouse liver cytosol was the most efficient in decreasing the AFB<sub>1</sub>-DNA binding. The cytosol from the bovine nasal olfactory mucosa had a somewhat lower activity and the lowest effect was seen by the bovine liver cytosol.

### Salmonella mutagenicity test

The *Salmonella* mutagenicity assay showed that S-9 fractions from both the nasal olfactory mucosa and the liver activated AFB<sub>1</sub> to a mutagenic metabolite. For all treatments the number of revertants obtained differed significantly ( $P < 0.001$ ) from the spontaneous number of revertants. At 100 μg protein/plate, increasing mutagenicity was seen for increasing concentrations of the AFB<sub>1</sub>, with preparations from the olfactory mucosa being much more effective than those from the liver (Figure 3). These results were confirmed and extended in experiments using different amounts of S-9 protein (Figure 4). It is well known that extensive metabolism of AFB<sub>1</sub> leads to the production of metabolites which are toxic to *Salmonella* bacteria, leading to a decreased number of revertants. This is clearly illustrated in Figure 4, where it can be seen that preparations from the olfactory mucosa at increasing tissue concentrations first induce an



**Fig. 3.** Effects of various amounts of AFB<sub>1</sub> on *S.typhimurium* mutagenicity mediated by S-9 preparations from bovine olfactory mucosa or liver, present in a concentration corresponding to 0.1 mg protein. All values are means ( $\pm$  SD) of revertants computed from the results of two separate experiments, each with three plates.



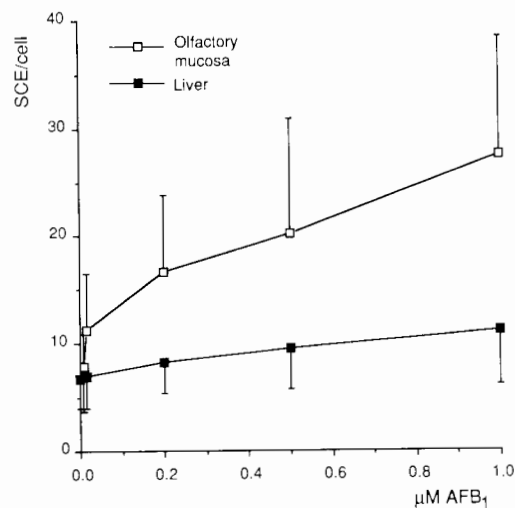
**Fig. 4.** Effect of 0.5  $\mu$ g of AFB<sub>1</sub> on *S.typhimurium* mutagenicity mediated by various amounts of S-9 preparations from bovine olfactory mucosa or liver. All values are means ( $\pm$  SD) of revertants computed from the results of two separate experiments, each with three plates.

increasing number of revertants, followed by a decreased number at high protein concentrations. For the preparation from the bovine liver with a lower metabolizing capacity, there was a slow increase in the number of revertants with increasing tissue concentration, without any signs of toxicity.

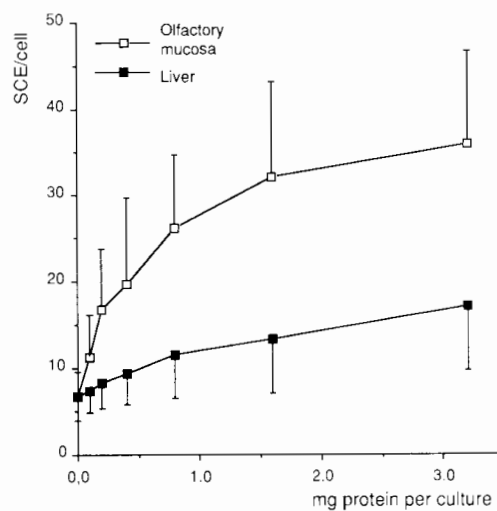
#### SCE assay

At a concentration of 2  $\mu$ M and above, AFB<sub>1</sub> treatments produced statistically significant ( $P < 0.05$ ) induction of SCE even in the absence of an S-9 fraction. At concentrations  $>50 \mu$ M the base-line level of SCE was more than doubled. However, at a concentration of 1  $\mu$ M and lower, AFB<sub>1</sub> was unable to induce SCE (data not shown).

The data in Figure 5 illustrate the efficiency of various concentrations of AFB<sub>1</sub> to induce SCE when the amount of S-9 preparation present during the treatment was fixed at 0.2 mg



**Fig. 5.** The frequency of SCE in CHO cells, obtained after a 1 h treatment with various doses of AFB<sub>1</sub> in the presence of S-9 preparations from bovine olfactory mucosa or liver in a concentration corresponding to 0.2 mg protein. Bars represent standard deviation of the mean.



**Fig. 6.** The influence of various amounts of S-9 preparation from bovine olfactory mucosa or liver, present during a 1 h treatment with 0.2  $\mu$ M AFB<sub>1</sub>, on the induction of SCE in CHO cells. Bars represent standard deviation of the mean.

protein. The S-9 preparation from the nasal olfactory mucosa was much more efficient than the S-9 preparation from the bovine liver to convert AFB<sub>1</sub> into mutagenic metabolites. Thus, while the nasal olfactory S-9 preparation induced a significant increase in the AFB<sub>1</sub>-induced SCE frequency at all concentrations tested (though only at the  $P < 0.05$  level at 0.01  $\mu$ M AFB<sub>1</sub>), the liver S-9 preparation promoted the induction of SCE only when the concentration of AFB<sub>1</sub> was 0.2  $\mu$ M or higher.

Figure 6 illustrates the influence of the amount of S-9 fraction on the yield of SCE induced by 0.2  $\mu$ M AFB<sub>1</sub>. The S-9 preparations of the nasal mucosa gave rise to a highly significant ( $P < 0.001$ ) induction of SCE at all S-9 concentrations tested. S-9 preparations from the liver were less potent in promoting the induction of SCE. Thus, there was no significant effect in the presence of 0.1 mg S-9 preparation. At 0.2 mg the increase was significant at the  $P < 0.01$  level, and at higher concentrations of the liver S-9 preparations the increases were highly significant ( $P < 0.001$ ).

## Discussion

As in our previous investigation (5), we find in the study presented here that the bovine nasal olfactory mucosa has a much higher capacity than the liver to bioactive AFB<sub>1</sub>. Thus, our present data showed that nasal microsomes had a higher ability than liver microsomes to induce covalent binding of AFB<sub>1</sub> to calf thymus DNA and to microsomal proteins. The major DNA adduct formed was found to be AFB<sub>1</sub>-N<sup>7</sup>-Gua. This adduct has been found to be the major AFB<sub>1</sub>-DNA adduct in all systems and is considered to be the one responsible for cell transformation and tumor development (1,10,13,21).

It has been reported that the nasal mucosa of a number of species contains cytochrome P450 enzymes and is capable of metabolizing many xenobiotics (28–31). Our previous study (5) reported that the level of cytochrome P450 in the bovine nasal mucosa was lower than in the bovine liver. However, the NADPH cytochrome *c* reductase activity and the cytochrome *b*<sub>5</sub>: cytochrome P450 ratio were highest in the nasal mucosa, conditions that may promote the bioactivation of AFB<sub>1</sub> in this tissue (5). Reed *et al.* (30) also showed a higher NADPH cytochrome *c* reductase: cytochrome P450 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. It has been demonstrated that the metabolism of AFB<sub>1</sub> in hepatic microsomes can be enhanced by cytochrome *b*<sub>5</sub> (32). The higher bioactivation of AFB<sub>1</sub> in the olfactory mucosa than in the liver may also be related to differences in the cytochrome P450 isoenzyme profile. It has been shown that the nasal mucosa of rabbits, rats and cattle contains unique forms of cytochrome P450, and these may have a high capacity to metabolize AFB<sub>1</sub> (33–35). We have found a higher metabolism of AFB<sub>1</sub> in the nasal mucosa than in the liver also in mice (36) and rats (P.Larsson and H.Tjälve, unpublished results).

Studies in rodents have indicated that enzymatic conjugation of the reactive AFB<sub>1</sub>-8,9-epoxide with GSH by the action of GST is a critical determinant for the hepatocarcinogenicity of AFB<sub>1</sub> (11,12,24,25). Mice are resistant to the hepatocarcinogenicity of AFB<sub>1</sub>, apparently due to highly efficient conjugation of the epoxide with GSH (11,12,24,25). The results of the present study indicate that GST activity towards bovine-olfactory-microsome-produced AFB<sub>1</sub>-8,9-epoxide is higher in the mouse liver than in the bovine olfactory mucosa. Therefore, a part of the AFB<sub>1</sub>-epoxide formed should be available for interaction with DNA in the bovine nasal mucosa. It has been shown that the AFB<sub>1</sub>-epoxide binds readily to double-stranded DNA (37). AFB<sub>1</sub> can also interact with DNA without activation and it has been proposed that intercalation is part of the AFB<sub>1</sub> binding process with DNA (37,38). We have previously shown that incubation of pieces of the bovine nasal olfactory mucosa with [<sup>3</sup>H]AFB<sub>1</sub> results in a preferential labelling of the nuclei of sustentacular cells and cells of the Bowman's glands (5). These cells probably have a high capacity to bioactivate AFB<sub>1</sub>, and the labelling of the nuclei may reflect a specific affinity of the bioactivated AFB<sub>1</sub> for the DNA.

Our results showed that incubation of 9000 *g* supernatant preparations of the bovine nasal olfactory mucosa with AFB<sub>1</sub> resulted in a strong genotoxic response both as regards induction of gene mutations in *S.typhimurium* and the induction of SCE in CHO cells. Preparations of the bovine liver (9000 *g*) had a much lower ability to induce these effects. Previous studies in rats with several naturally occurring aflatoxins have shown that there is a correlation between the hepatocarcinogenic potential

of these substances and their ability to act as mutagens in the hepatic microsome-mediated *S.typhimurium* mutagenicity assay (39,40). Strain TA100 of *S.typhimurium*, which was used as an indicator organism in our study, preferentially detects the base pair substitution type of mutations (26). There is evidence that base pair substitutions are the main biochemical events responsible for the AFB<sub>1</sub>-induced mutagenicity (41). Thus, the formation of AFB<sub>1</sub>-N<sup>7</sup>-Gua adducts can lead to mispairings with A opposite the AFB<sub>1</sub>-substituted sites and the end-result can be G-C to T-A transversion. It has been proposed that this type of transversion may result in activation of proto-oncogenes and underly the AFB<sub>1</sub>-induced liver tumorigenesis (41).

It has been shown that a high degree of bioactivation of AFB<sub>1</sub> results in toxic effects towards *S.typhimurium* (42). In our experiments it was found that AFB<sub>1</sub>, assayed with high levels of protein in S-9 mix derived from nasal olfactory mucosa, was toxic to *S.typhimurium*. This clearly demonstrates the higher capacity of the olfactory mucosal S-9 fraction to bioactivate AFB<sub>1</sub>, since no reduction in revertants was observed when liver S-9 fraction was used.

SCE is a sensitive genetic indicator of many types of DNA damage in mammalian cells. Several studies with CHO cells, Chinese hamster line V79 cells and human lymphocytes, in which hepatic 9000 *g* supernatants were used for promutagen activation, have shown that the SCE test is sensitive in detecting the genotoxic potential of AFB<sub>1</sub> (43–46). In addition to the efficient induction of SCE in the presence of the 9000 *g* supernatants, we found that high doses of AFB<sub>1</sub> without activation increased the number of SCE in our CHO cell line. Similar results have been reported previously (43,44). The mechanism underlying this effect is not known. However, considering the reports that AFB<sub>1</sub> can bind to DNA without activation via a mechanism which may involve intercalation (37,38) one may propose that this type of interaction may cause the SCE induction at high AFB<sub>1</sub> doses. Another possibility is that the CHO indicator cells during propagation *in vitro* have retained some enzymatic potential to convert AFB<sub>1</sub> into its active metabolite.

As mentioned in the Introduction, nasal olfactory tumors occur relatively frequently in cattle in several developing countries (6–8). Since affected animals sometimes show signs of severe aflatoxicosis (6) a role of AFB<sub>1</sub> in tumorigenesis can be proposed. Our present and previous (5) studies support this assumption. Bioactivation of AFB<sub>1</sub> has been shown in cultured tracheal epithelium of rats, hamsters and monkeys (47). Rats given AFB<sub>1</sub> intratracheally have been shown to develop tracheal tumours (48). Thus, local exposure of a tissue with AFB<sub>1</sub>-bioactivating capacity may promote tumorigenesis at that site. Cattle may inhale high concentrations of AFB<sub>1</sub> via respirable feed-dust particles (3,4) leading to a high exposure of the nasal epithelium, and this may increase the risk of carcinogenesis of this tissue.

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