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# DIETARY CHOLESTYRAMINE REDUCES OCHRATOXIN A-INDUCED NEPHROTOXICITY IN THE RAT BY DECREASING PLASMA LEVELS AND ENHANCING FECAL EXCRETION OF THE TOXIN

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# DIETARY CHOLESTYRAMINE REDUCES OCHRATOXIN A-INDUCED NEPHROTOXICITY IN THE RAT BY DECREASING PLASMA LEVELS AND ENHANCING FECAL EXCRETION OF THE TOXIN

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*Ochratoxin A (OTA) is a mycotoxin that may contaminate animal feed (oat, barley, and rye) and food (wheat, rice, coffee, beer, pig meat), leading to major health problems (e.g., nephropathy) in several animal species including humans. Several methods have been tested to reduce the toxicity of OTA in animals but with limited success. In rats, the effect of cholestyramine (CHA), a bile acid-binding resin, was investigated on OTA-induced nephrotoxicity and bioavailability. Animals were fed semisynthetic diets containing two levels of OTA: 1 or 3 ppm. At each level of OTA, the diets were enriched with 0.1, 1, and 5% of CHA. The results showed that CHA decreased the concentration of OTA in plasma. At 1 and 3 ppm of OTA in the diet, CHA is effective at a level of 0.1% and 5%, respectively. The excretion of OTA and its metabolites (ochratoxin alpha and hydroxylated ochratoxin A) in bile and urine was also decreased by addition of 5% CHA in the diet. This was associated with an increase of OTA excretion in feces. Enzymuria and renal morphology revealed that dietary CHA can decrease OTA-induced nephrotoxicity, probably by reducing renal exposure to the toxin. In conclusion, CHA can reduce OTA concentrations in plasma as well as reducing nephrotoxicity, which may be attributed to a decrease of bioavailability and/or enterohepatic circulation of the toxin.*

Ochratoxin A (OTA) is a mycotoxin produced by fungi such as *Penicillium verrucosum* (Frisvad & Samson, 1991) and *Aspergillus ochraceus* (De Scott, 1965; Frisvad & Samson, 1991). OTA may be contained in various grains, cereals, animal feeds, meats, and may be found in blood of animals and humans after consumption of contaminated food (Krogh,

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1987; Petkova-Bocharova et al., 1988; Kuiper-Goodman & Scott, 1989; Creppy et al., 1991; Maaroufi et al., 1995; Marquardt et al., 1990). OTA can induce nephrotoxicity in various animal species (Krogh et al., 1974, 1979; Kuiper-Goodman & Scott, 1989), and is possibly involved in the Balkan endemic nephropathy in humans (Krogh, 1987). It has also been shown that OTA is an immunosuppressor (Creppy et al., 1983; Lea et al., 1989), a teratogen (Mayura et al., 1984), a genotoxin (Kane et al., 1986b; Pfohl-Leszkowicz et al., 1991; Creppy et al., 1985), and a carcinogen (Kanisawa & Suzuki, 1978; National Toxicology Program, 1989).

OTA is of concern because there is a high potential for its production in stored foods and animal feeds under appropriate temperature and moisture conditions. The public health implications of food contamination with OTA and the difficulties in controlling toxicogenic moisture require the development of means for its rapid elimination from the body and for reduction of its absorption, and, in time, of its toxic effects (Baudrimont et al., 1995). Several methods have been tested, including extraction, heat treatment (Josefsson & Moller, 1980), ammoniation (Chelkowski et al., 1981), ensiling (Rotter et al., 1990), and increasing the dietary protein or phenylalanine intake (Bailey et al., 1989, 1990; Rotter et al., 1989a). Nonspecific adsorbants, such as charcoal, have also been tested (Rotter et al., 1989b). Although some of these methods have shown promise, most have only a minor effect on the tolerance to, or the disposition of, OTA in animals (Marquardt & Frohlich, 1992).

Recent studies have indicated that cholestyramine (CHA), an ion-exchange resin with strong affinity for bile salts (Kos et al., 1991), can decrease plasma concentration of OTA and increase its fecal excretion in the rat (Madhyastha et al., 1992). The aim of this study was to determine if dietary CHA can prevent OTA-induced nephrotoxicity, and whether such an effect is related to lower bioavailability of the toxin.

## MATERIALS AND METHODS

### Animals

Male Sprague-Dawley rats (Charles River, St. Constant, Quebec, Canada) weighing 170–180 g were randomly divided into 13 groups of 6 animals each and placed individually in metabolic cages. Animals were maintained under constant temperature (22°C) and lighting (7 p.m. to 7 a.m. darkness) with free access to a semisynthetic diet (AIN-76A) and water ad libitum.

### Treatment

Ochratoxin and 4-*R*-hydroxyl ochratoxin were produced using solid-phase fermentation of wheat inoculated with *Aspergillus ochraceous* NRRL 3174 followed by extraction and purification as described by Xiao

et al. (1995, 1996). Ochratoxin α was synthesized by acid hyrolysis of OTA according to van der Merwe et al. (1965). The compounds were shown to be analytically pure as determined from high-performance liquid chromatography (HPLC) analysis using fluorescence and photodiode array ultraviolet detection, melting points, mass spectrometry, and nuclear magnetic resonance (NHR) spectrometry. CHA was purchased from Sigma Chemical Co. (St. Louis, MO). The semisynthetic diets contained 2 levels of OTA: 1 or 3 ppm (mg/kg). A first series of experiments was conducted with animals fed diets supplemented with 0.1, 1, and 5% CHA (the quantity of CHA replaced the same amount of sucrose in the diets). To determine the minimum effective CHA concentrations, a second series of experiments has been carried out. At a level of 1 ppm OTA, diets were supplemented with 0.025 and 0.06% CHA. At 3 ppm OTA, diets were supplemented with 2.5 and 3.5% resin.

After an acclimatization of 3 d to the control diet (basal diet without OTA or CHA), rats were fed experimental diets containing OTA alone, CHA alone, or OTA plus CHA in different concentrations for a period of 14 d.

Under ether anesthesia, blood samples were collected from the retro-orbital sinus on d 2, 7, 10, and 14 to determine OTA concentration in plasma. Urine and feces were collected daily from d 7 to 14. After measurement of urine volume and feces weight, samples were frozen and stored at –20°C pending analysis of OTA and its metabolites ochratoxin alpha (OTα) and hydroxylated ochratoxin A (HO-OTA).

On d 14, the rats were anesthetized with sodium pentobarbital (55 mg/kg body weight, ip), and the common bile duct was cannulated with a PE-10 catheter. Bile was collected on ice in preweighed tubes during 1 h at 15-min intervals. Body temperature was maintained throughout at 37 ± 1°C, using a rectal probe and thermostatically controlled heat lamp. Bile volume was determined gravimetrically, assuming a density of 1 g/ml.

The rats were sacrificed by exsangination via the abdominal aorta, and pieces of liver and right kidney were fixed for further histological examination.

### Analytical Methods

OTA and its metabolites (OTα, HO-OTA) were determined on d 2, 7, 10, and 14 in plasma, urine, and feces, and in bile only on d 14. The same extraction procedure (Hult et al., 1979) was used to measure OTA and its metabolites in the diets, blood, serum, and feces. One hundred microliters of serum was used for extraction. Fecal samples were dried/lyophilized for 48 h prior to extraction. One gram of feces was homogenized with 10 ml of HCl:$MgCl_2$ solution (as in the case of serum), its pH was adjusted to 2.3 with 6 *N* HCl, and it was then extracted with 6 ml chloroform. Bile and urine samples were mixed 1:4 methanol, centrifuged at 14,000 × g for 15 min, and injected directly into the C18 col-

umn. The HPLC analyses were performed according to Frohlich et al. (1997) using a gradient mobile phase that consisted of double-distilled water adjusted to pH 2.1 with $H_3PO_4$ (solvent A) and methanol containing 10% isopropanol (solvent B) at a flow rate of 1.5 ml/min and a temperature of 40°C. The amount of solvent B in the mobile phase was increased linearly from 30 to 48% in the first 10 min of the run, kept constant at 48% for 6 min, and increased to 70% over 9 min. During this time period all detectables compounds were eluted. The column was washed with 90% solvent B for 6 min and further equilibrated with 30% solvent B for 10 min. Ten-microliters aliquots of samples were injected using an automatic injector (Waters WISP 712). The HPLC system consisted of an LKB pump 2150, controller 2152, and column oven 2155.

Ochratoxin A and its metabolites were detected with a Shimadzu RF 545 fluorescence detector set at 333 nm for excitation and at 450 nm for emission. Pure ochratoxin A and 4-*R*-hydroxy-ochratoxin A were prepared as described by Xiao et al. (1995, 1996). Ochratoxin α was prepared according to van der Merwe (1965). Fecal, blood, urine, and bile samples from non-OTA-treated animals that were extracted as already described did not have peaks that coeluted with OTA, OTA, or HO-OTA. Hydrolysis of OTA to OTα and hydroxy-OTA to its hydroxylated isocoumarin moiety yielded peaks that coeluted with the corresponding standards. Studies with the diets, blood, urine, bile, and feces when spiked with OTA, OTα, and HO-OTA yielded recoveries of from 95 to 105%.

### Biochemical Assays

Several assays were used to evaluate the potential protective effect of cholestyramine on renal damage induced by OTA. To evaluate nephrotoxicity, enzymuria, creatinine, and glucose were determined in urine. Gamma-glutamyl transferase (GGT, EC 2.3.2.2) activity was assessed by a modification of the methods of Szasz (1969) and Dierickx (1981). *N*-Acetyl-β-D-glucosaminidase (NAG, EC 3.2.1.30) was determined by the method of Horak et al. (1981). Glucose and creatinine in urine were determined using Sigma kits numbers 115-A and 555-A, respectively. Total bile acids in bile and in feces were assessed with 3-α-hydroxysteroid dehydrogenase according to the method of Weber et al. (1972). For evaluation of possible liver damage, serum alanine aminotransferase (ALT, EC 2.6.1.2) and aspartate aminotransferase (AST, EC 2.6.1.1) activities were assessed using the sigma kit number 505-OP. The triglyceride content of livers was determined using a Boehringer Mannheim kit (GPO-PAP, number 701882, Montreal, Quebec, Canada) following lipids extraction.

### Histopathology

Sections of renal and hepatic tissue were collected for light microscopy. Kidney and liver tissue were fixed in alcohol–formol solution (20

ml of 10% formalin-buffered solution, 80 ml anhydrous ethanol), and paraffin-embedded sections were stained with hematoxylin–phloxine.

### Statistics

The data were evaluated by one-way analysis of variance (ANOVA), and when values were found to be significant, comparisons between groups were made using Duncan's test. All results are expressed as means ± standard error. Differences were considered to be statistically significant at $p < .05$.

## RESULTS

The addition to the diet of OTA or CHA alone or in combination did not influence food intake, body weight gain, or liver and kidney weight (results not shown). Table 1 presents results of urinary and fecal output. The addition of CHA to the diet did not change the urine output. The feces weight was increased significantly by the addition of 2.5 and 5% CHA, either in groups receiving or not OTA.

### Ochratoxin A Concentration in Plasma

Figure 1 shows the effect of CHA on OTA concentrations in plasma. As expected, animals fed diets containing 3 ppm OTA exibited a higher plasma level of the toxin ($\chi^2$) when compared to animals fed diet contain-

**TABLE 1.** Effect of CHA on the urinary volume and the fecal weight from rats fed a diet containing 1 or 3 ppm of the mycotoxin

| OTA (ppm) | CHA (g/100 g) | Urinary volume[a] (ml) | Fecal weight[a] (g) |
|---|---|---|---|
| 0 | 0 | 9.2 ± 2.7 | 1.5 ± 0.3 |
| 0 | 0.1 | 9.7 ± 2.8 | 2.0 ± 0.1 |
| 0 | 2.5 | 8.0 ± 3.2 | 3.0 ± 0.3[b,c] |
| 0 | 5 | 9.0 ± 1.2 | 3.7 ± 0.5[b,c] |
| 1 | 0 | 12.3 ± 2.2 | 1.9 ± 0.1 |
| 1 | 0.1 | 18.3 ± 4.5 | 2.0 ± 0.1 |
| 1 | 1 | 10.3 ± 1.8 | 2.5 ± 0.1 |
| 1 | 5 | 12.6 ± 2.3 | 4.6 ± 0.5[b,c] |
| 3 | 0 | 16.4 ± 3.0 | 2.0 ± 0.2 |
| 3 | 0.1 | 11.0 ± 1.6 | 2.0 ± 0.4 |
| 3 | 1 | 11.7 ± 2.9 | 2.0 ± 0.3 |
| 3 | 2.5 | 9.7 ± 5.8 | 3.3 ± 0.3[b,c] |
| 3 | 5 | 9.9 ± 1.3[c] | 5.1 ± 0.4[b,c] |

[a]Daily mean (d 11–14), values expressed as mean ± SEM (5–6 rats/group).
[b]Significantly different from control group, $p < .05$.
[c]Significantly different from their respective group treated with OTA alone (at 1 ppm or 3 ppm) $p < .05$.

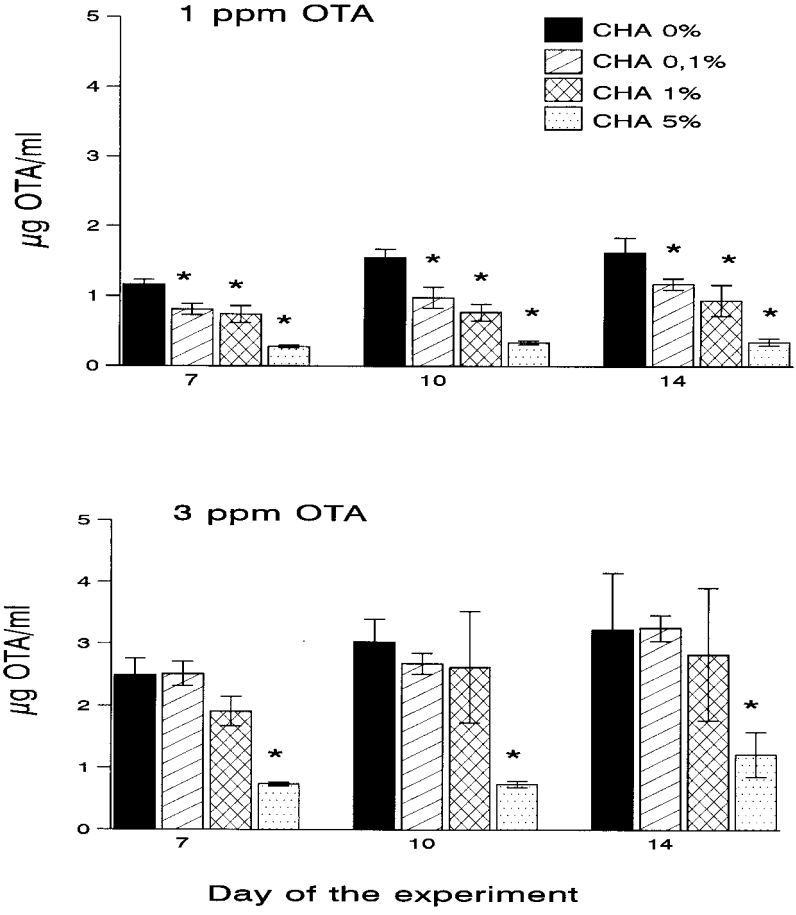



**FIGURE 1.** Effect of cholestyramine (CHA) on plasma concentration of ochratoxin A (OTA) in rats fed diet containing 1 and 3 ppm of the mycotoxin. Plasma OTA concentration was decreased by addition of the resin in a dose-dependent manner. Bars represent the means ± standard error of five to six rats per group. Asterisk indicates significantly different from the group treated with OTA alone (without CHA), $p < .05$.

ing 1 ppm. At both levels of the mycotoxin, a steady state of plasma concentration of OTA was obtained between d 7 and 10.

When CHA is added to diets containing OTA, a lower plasma level of the toxin is observed. At 1 ppm (1 mg/kg) OTA in the diet, CHA significantly decreased OTA concentrations in plasma at all levels examined. Average of the decreases were 30, 36, and 70% for 0.1, 1, and 5% CHA, respectively, at each time period of study. CHA concentrations lower than 0.1% did not significantly reduce the plasma level of the toxin (1.6 ± 0.05 µg/ml vs. 1.23 ± 0.14 µg/ml) for the control and 0.06% CHA diets, respectively.

At 3 ppm OTA in diet, a level of 5% CHA was required to decrease

significantly the OTA plasma concentration, and the reduction was about 70% at each time period of observation (Figure 1). In the additional study using CHA concentrations between 1 and 5%, it was shown that at least 2.5% of the resin was necessary to decrease significantly OTA concentration in plasma (4.1 ± 0.3 μg/ml vs. 1.6 ± 0.5 μg/ml) for the control and 2.5% CHA, respectively.

At 1 and 3 ppm OTA, the metabolite OTα was detected in plasma but its concentration was low (mean < 0.016 μg/ml for all time periods examined). The addition of CHA did not markedly change the concentration of OTα in plasma (results not shown).

### Secretion of OTA and Its Metabolites in Bile

Bile flow was virtually similar in all treated groups (0.95–1.22 μl/min/g liver) and did not differ significantly from control groups (without OTA or CHA). Table 2 shows secretion rates of OTA and its metabolites in bile. In the groups treated with OTA alone (given 1 or 3 ppm OTA), the toxin was mainly secreted as OTA, with low secretion rates of OTα (nontoxic metabolite) and HO-OTA (as toxic as OTA). In animals fed diets containing 3 ppm OTA, the secretion rates of the toxin and its metabolites in bile were more than twice those seen in rats fed 1 ppm OTA.

The secretion rate of OTA in bile of rats fed diets containing 1 ppm OTA was significantly reduced when diets were supplemented with 1% or 5% CHA. At 3 ppm OTA, the secretion rates of the mycotoxin and its metabolites (OTα and HO-OTA) in bile were significantly reduced when diets contained 1 and 5% CHA.

### Excretion of OTA and Its Metabolites in Urine

Figure 2 indicates that the urinary secretion rates of OTA and its metabolites were 2.5 times higher in the group fed 3 ppm of the toxin

**TABLE 2.** Effect of CHA on the excretion of OTA, OTα, and HO-OTA in bile from rats fed a diet containing 1 or 3 ppm of the mycotoxin

| OTA (ppm) | CHA (g/100 g) | OTA[a] (ng/h/g liver) | OTα[a] (ng/h/g liver) | HO-OTA[a] (ng/h/g liver) |
|---|---|---|---|---|
| 1 | 0 | 5.10 ± 0.99 | 0.58 ± 0.21 | 0.83 ± 0.38 |
| 1 | 0.1 | 5.47 ± 1.41 | 0.56 ± 0.21 | 0.10 ± 0.10 |
| 1 | 1 | 2.97 ± 0.68[b] | 0.23 ± 0.11 | 0.35 ± 0.23 |
| 1 | 5 | 1.43 ± 0.44[b] | 0.38 ± 0.23 | nd |
| 3 | 0 | 12.43 ± 4.30 | 1.2 ± 0.34 | 3.03 ± 1.36 |
| 3 | 0.1 | 14.20 ± 2.60 | 1.54 ± 1.11 | 2.10 ± 0.84 |
| 3 | 1 | 6.60 ± 1.66 | 0.15 ± 0.03[c] | 1.05 ± 0.40[c] |
| 3 | 5 | 3.83 ± 0.85[c] | 0.04 ± 0.02[c] | 0.43 ± 0.26[c] |

[a]Values expressed as means ± SEM (4–5 rats); nd, not detected.
[b]Significantly different from the group treated with 1 ppm OTA, $p < .05$.
[c]Significantly different from the group treated with 3 ppm OTA, $p < .05$.

than in the group fed 1 ppm. For both levels of OTA, the toxin was excreted in the urine mainly as OTα and to a lesser degree as OTA and HO-OTA. It was noted that the level of OTα excreted in urine decreased with time after reaching a maximal value on d 10.

With the addition of 5% CHA, a significant decrease of HO-OTA was seen but only on d 7. On the other hand, OTα excretion was significantly decreased on d 7 and 10 by the addition of CHA at a level of 0.1, 1, and 5%. After feeding 3 ppm OTA, all levels of CHA reduced significantly the excretion of OTA but only on d 14. Finally, CHA at concentrations of 1 and 5% significantly decreased the excretion of OTα on d 7, 10, and 14.

### Excretion of OTA and Its Metabolites in Feces

Figure 3 shows that the excretion of OTA and its metabolites in feces was significantly higher in the group fed 3 ppm OA than in the 1 ppm OA

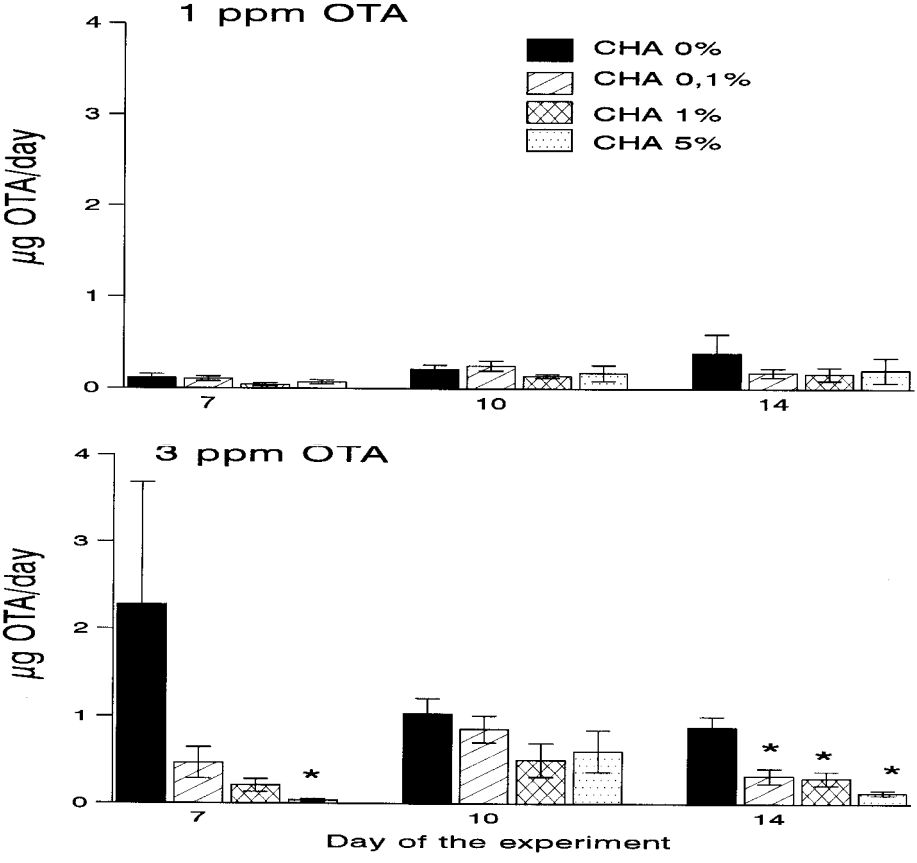
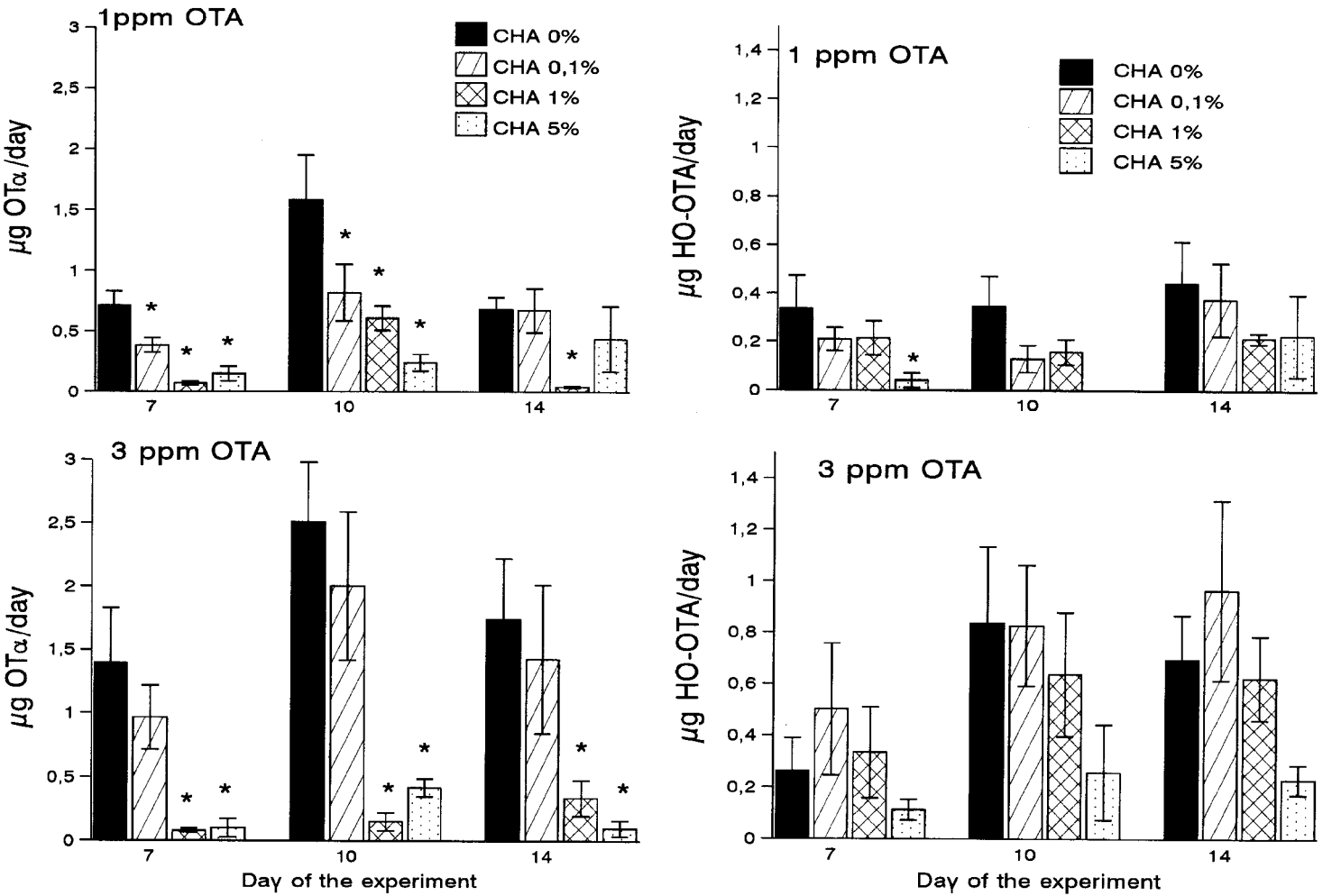



**FIGURE 2.** Effect of CHA on urinary excretion of OTA, OTα, and HO-OTA. Bars represent the means ± standard error of five to six rats per group. Asterisk indicates significantly different from the group treated with OTA alone (without CHA), $p < .05$.


1ppm OTA
CHA 0%
CHA 0,1%
CHA 1%
CHA 5%
µg OTα/day
1 ppm OTA
µg HO-OTA/day
3 ppm OTA
Day of the experiment

group. At 1 ppm OTA, the maximal excretion of OTA and its metabolites was reached on d 10. The addition of CHA at a level of 1 and 5% significantly increased the fecal excretion of OTA on d 7 and 10. On d 14, only CHA at 5% increased the OTA output. CHA at levels of 0.1, 1, and 5% significantly decreased the excretion of the metabolite OTα in feces on d 10. On d 7 and 14, CHA only at 5% lowered OTα excretion significantly. With 3 ppm OTA, CHA at levels of 1 and 5% significantly increased the excretion of OTA on d 10 and 14, and it significantly decreased OTα excretion at all times examined.

### Excretion of Total Bile Acids in Bile and Feces

As shown in Table 3, the addition of CHA alone to the diet significantly decreased the total bile acids excreted in bile and increased their excretion in feces. This effect was observed with all doses tested. It was

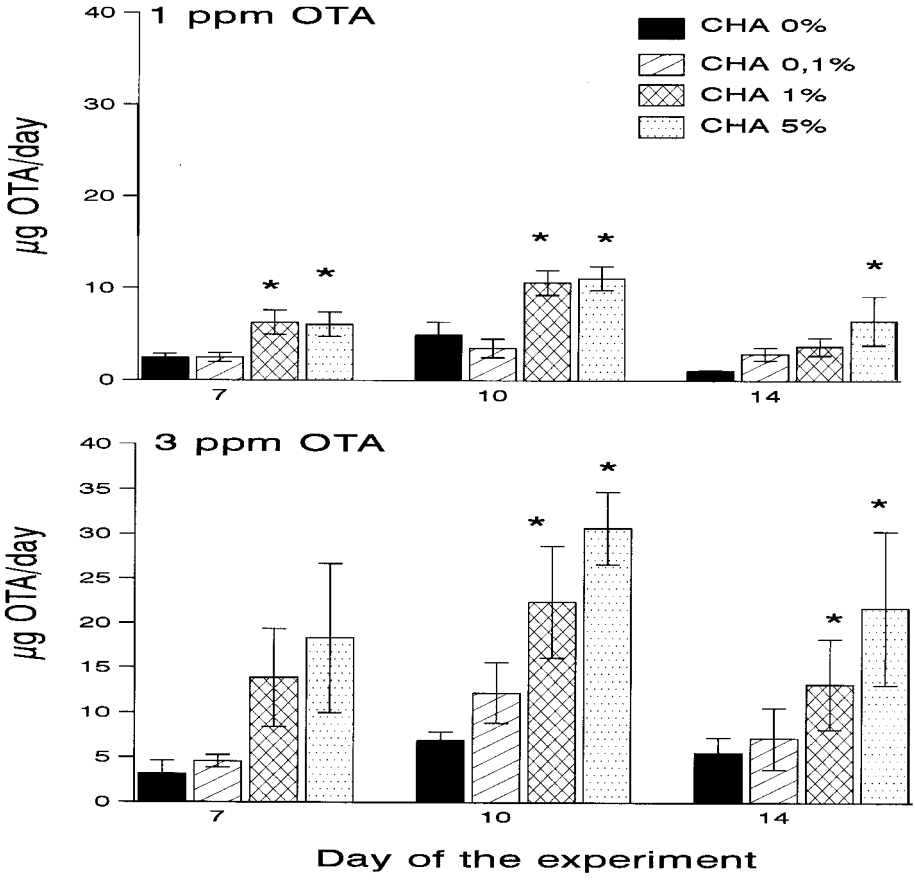
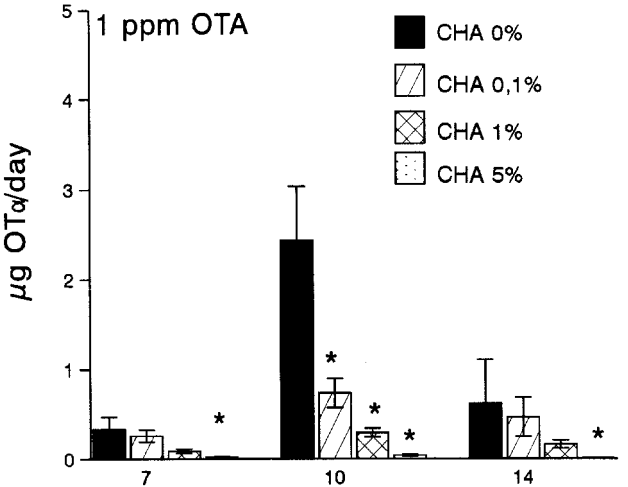
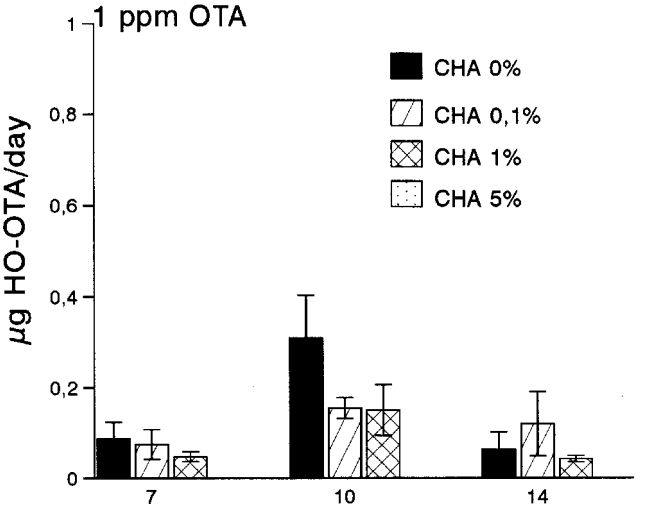
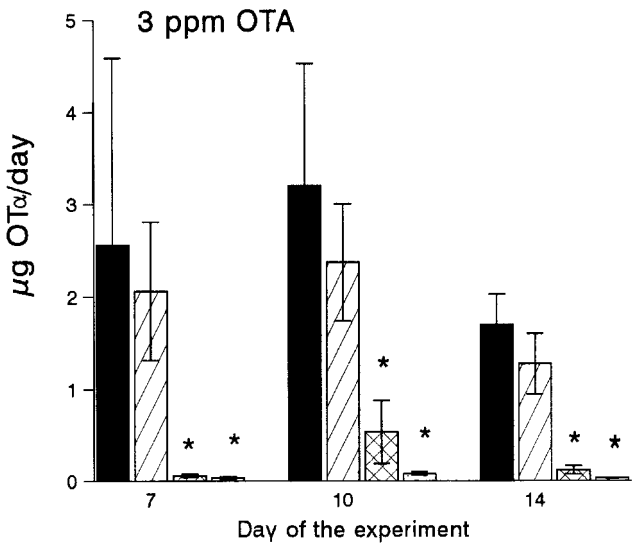



**FIGURE 3.** Effect of CHA on excretion of OTA, OTα, and HO-OTA in feces. Bars represent the means ± standard error of five to six rats per group. Asterisk indicates significantly different from the group treated with OTA alone (without CHA), $p < .05$.


1 ppm OTA
CHA 0%
CHA 0,1%
CHA 1%
CHA 5%
µg OTα/day
0
1
2
3
4
5
7
10
14
*


1 ppm OTA
CHA 0%
CHA 0,1%
CHA 1%
CHA 5%
µg HO-OTA/day
0
0,2
0,4
0,6
0,8
1
7
10
14


3 ppm OTA
µg OTα/day
0
1
2
3
4
5
7
10
14
Day of the experiment
*

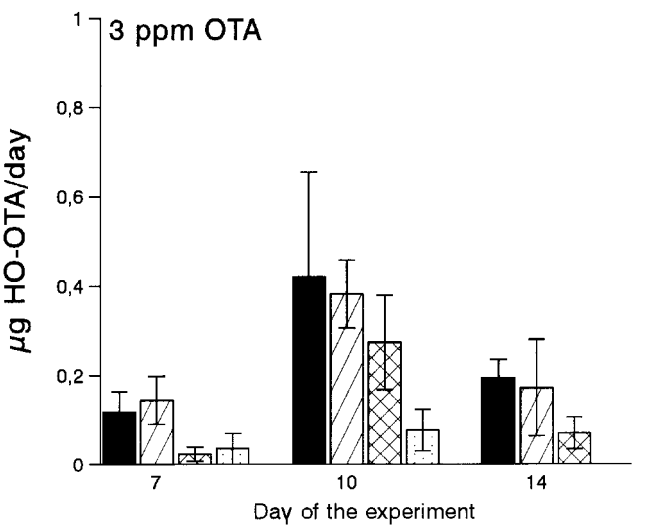

3 ppm OTA
µg HO-OTA/day
0
0,2
0,4
0,6
0,8
1
7
10
14
Day of the experiment

**TABLE 3.** Effect of CHA on the excretion of total bile acids in bile and feces in rats fed a diet containing 1 or 3 ppm of the mycotoxin

| OTA (ppm) | CHA (g/100 g) | Excretion in bile[a] (μmol/d) | Excretion in feces[a] (μmol/d) |
|---|---|---|---|
| 0 | 0 | 700 ± 104 | 9 ± 2 |
| 0 | 0.1 | 489 ± 104[b] | 43 ± 5[b] |
| 0 | 2.5 | 279 ± 19[b] | 43 ± 5[b] |
| 0 | 5 | 165 ± 12[b] | 115 ± 5[b] |
| 1 | 0 | 664 ± 134 | 12 ± 2 |
| 1 | 0.1 | 564 ± 71 | 16 ± 1 |
| 1 | 5 | 205 ± 12[b,c] | 99 ± 32[b,c] |
| 3 | 0 | 699 ± 56 | 27 ± 6[b] |
| 3 | 2.5 | 377 ± 31[b,c,d] | 39 ± 2[b,c] |
| 3 | 5 | 204 ± 70[b,c] | 114 ± 12[b,c] |

[a]Values expressed as means ± SE ($n$ = 3–4); bile and feces were collected on d 14.
[b]Significantly different from the control group, $p < .05$.
[c]Significantly different from group treated with OTA alone, $p < .05$.
[d]Significantly different from the group treated with CHA at 2.5%, $p < .05$.

noted that the addition of OTA at 1 or 3 ppm did not affect the biliary and fecal excretion of bile acids. In groups fed 1 ppm OTA, CHA treatment significantly decreased bile acid secretion in bile and increased fecal excretion only at a level of 5%.

At 3 ppm OTA, CHA treatment significantly affected bile acids excretion at both levels of the resin. The biliary bile acid output in rats fed diet containing 3 ppm OTA and CHA was higher than in rats fed diet with CHA alone, indicating that interactions between the mycotoxin and CHA may have occurred affecting bile acid excretion.

### Enzymuria, Kidney Function, and Morphology

For this study we have used diets containing 3 ppm OTA and 5% of CHA. The choices of these conditions were based on pilot histologic and functional studies. Data indicate that 1 ppm OTA did not result in significant kidney injury, while the 3 ppm dose caused nephrotoxicity.

The activities of GGT and NAG were measured in order to follow the subchronic renal intoxication by OTA and the possible protective effect of CHA. The results show that 2 wk of ingestion of diet containing 3 ppm OTA significantly increased enzymuria compared to the control animals (Figure 4). Urinary enzyme activities were increased by 90% and more for GGT on d 7 and 14. However, NAG activity remained in the normal range on d 7, and only increased from wk 2. This increase, in comparison with the controls, was approximately 60%. Animals treated with CHA alone for 2 wk did not show any modifications of enzymuria (31.9 ± 5.8 vs. 24.2 ± 3.3 IU/L for NAG and 37.5 ± 7.1 vs. 45.2 ± 10.2 IU/L for

GGT). In animals fed diets supplemented with CHA at 5%, the OTA-induced elevation of GGT and NAG activities was completely prevented by the resin. Furthermore, the values were not significantly different from the untreated controls (without OTA).

Concerning kidney function (Table 4), OTA induced a significantly decrease in creatinuria as compared with the untreated control animals. Similar results have been reported by Kane et al. (1986a) and Baudrimont et al. (1995). In animals given the OTA/CHA combination, creatinuria increased but it was not statistically significantly different from corresponding controls. Glucosuria was not significantly altered by OTA or additional CHA.

Figure 5 illustrates the light microscopic appearence of rat kidney

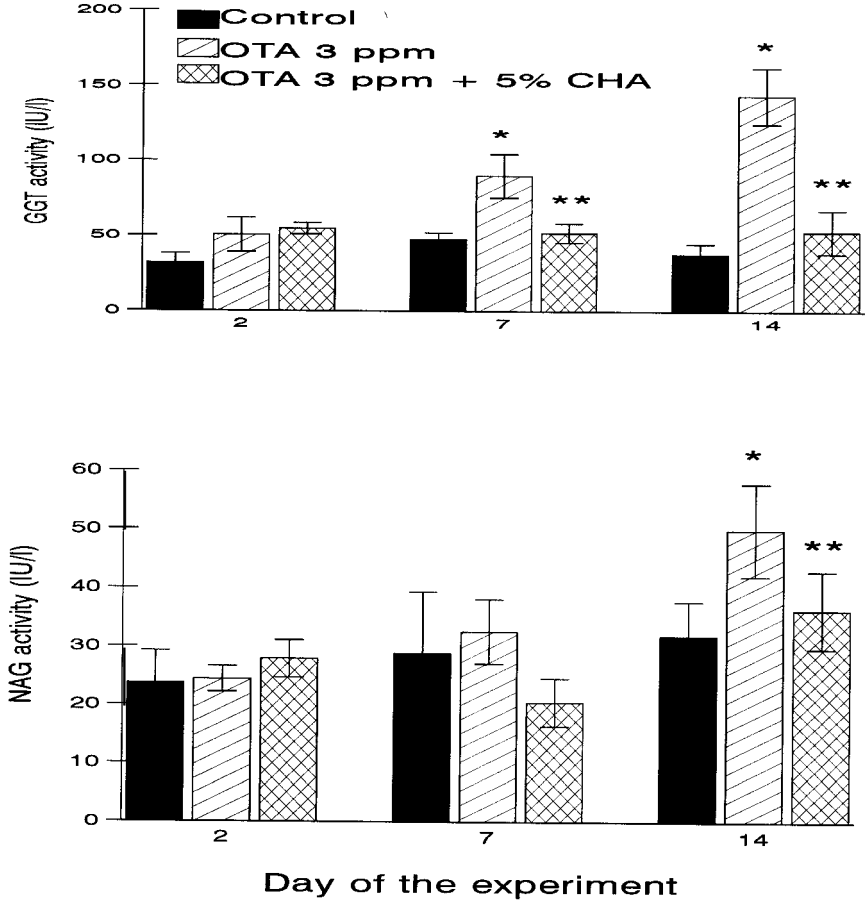



**FIGURE 4.** Effect of CHA on urinary activities of GGT and NAG in rats fed diet containing 3 ppm OTA. CHA protected animals from OTA-induced enzymuria. Bars represent the means ± standard error of five to six rats per group. Asterisk indicates significantly different from control group (without OTA or CHA), $p < .05$; double asterisk, significantly different from OTA group, $p < .05$.

**TABLE 4.** Effect of CHA on the excretion of creatinine and glucose in the urine from rats fed a diet containing 3 ppm of the mycotoxin

| Treatment | Creatinuria (mg/kg body weight) | Glucosuria (mg/kg body weight) |
|---|---|---|
| Control[a] | 23.2 ± 5.0 | 16.4 ± 3.0 |
| OTA, 3 ppm | 14.7 ± 1.2[b] | 11.8 ± 1.2 |
| CHA, 5% | 23.2 ± 5.2 | 15.9 ± 2.3 |
| OTA, 3 ppm, + CHA, 5% | 17.5 ± 2.5 | 18.1 ± 1.4 |

*Note.* Values expressed as mean ± SEM ($n$ = 4–6).
[a]Untreated control without OTA or CHA.
[b]Significantly different from the control group, $p < .05$.

after feeding a diet containing 3 ppm OTA. We observed damage to the proximal tubules, which are the main target segment of the nephron in OTA-induced nephropathy (Figure 5A). When CHA was present in the diet at 5%, normal renal morphology was observed (Figure 5B).

### Hepatic Function and Morphology

As previously mentioned, liver weight, hepatic macroscopic appearance, and bile flow and were not influenced by any of the treatments. However, histological examination (not shown) revealed that CHA (5%), OTA at 3 ppm, and the combined treatment (3 ppm OTA + 5% CHA) induced a slight vacuolization of liver parenchymal cells in the periportal area (not shown). The nature of these vacuoles seems to be hydropic, since the triglyceride content of livers did not differ markedly between groups (untreated control, 8.5 mg/g liver; 5% CHA, 10.1 mg/g liver; 3 ppm OTA, 11.4 mg/g liver; 5% CHA + 3 ppm OTA, 12.0 mg/g liver).

Although serum AST activity did not change with the different treatments, a slight elevation of ALT activity was observed in the diets containing 5% CHA as well as 3 ppm OTA + 5% CHA (untreated control, 19 ± 3; 5% CHA, 45 ± 2; 3 ppm OTA, 15 ± 2; 5% CHA + 3 ppm OTA, 41 ± 1 IU/L), suggesting an enhanced liver cell membrane permeability when 5% of CHA was added to the diet.

## DISCUSSION

The results of this study clearly demonstrate that CHA is effective in decreasing the plasma concentrations of OTA and that the concentration of CHA required to induce this effect depends on the amount of OTA contamination of food. At 1 ppm OTA the minimal effective level of CHA was 0.1%, and at 3 ppm OTA it was 2.5%. The lowered OTA concentration in plasma was associated with a prevention of OTA-induced nephrotoxicity as evaluated by output of urinary enzymes (GGT and NAG) and histologic examination.

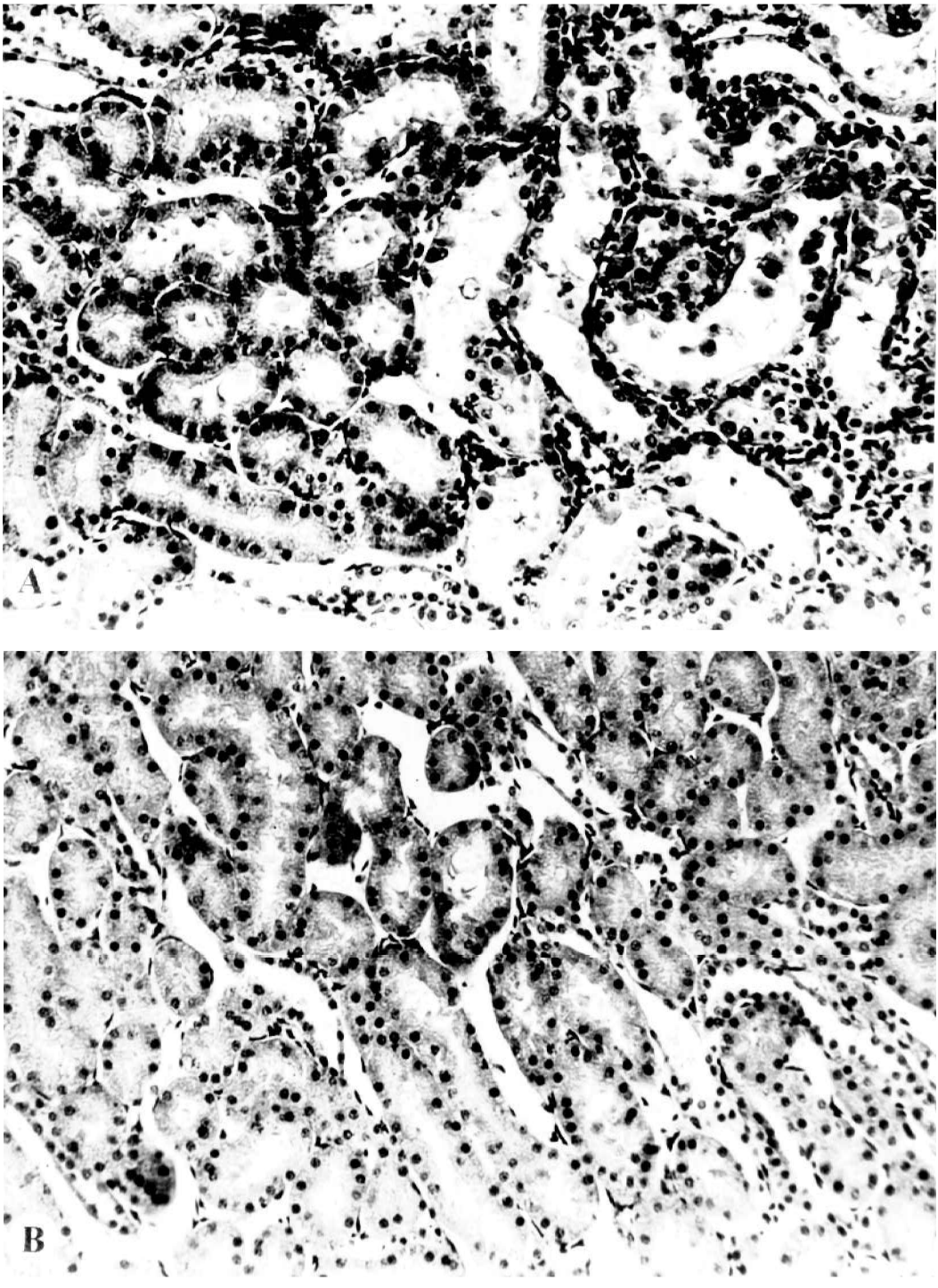



**FIGURE 5.** Light microscopy of kidney from rat fed diet containing 3 ppm of OTA for 14 d, ×200. (*a*) Without CHA, proximal renal tubules were affected by the toxin. (*b*) With addition of 5% CHA, kidney appeared virtually normal.

After ingestion OTA is absorbed in a passive manner from the jejunum in the non- or partially ionized form (Kumagai, 1988). Most OTA is bound to plasma albumin, and this not only facilites the passive absorption of the nonionized form but also delays OTA elimination and consequently contributes to the prolonged half-life of the toxin (Marquardt & Frohlich, 1992). In addition, OTA undergoes enterohepatic circulation (Roth et al., 1988; Fuchs et al., 1988), and this leads to overexposure of susceptible target organs, such as the kidneys, to the toxin. OTA undergoes rapid hydrolysis into dihydroisocoumarine (OTα) (Galtier, 1991), which is considered to be nontoxic to animals (Chu, 1974), and phenylalanine. It has been shown that this cleavage occurs in the small and large intestine via microbial activity (Galtier, 1991). Also, a small percentage of OTA absorbed is converted to hydroxyochratoxin A (HO-OTA) by both liver and kidney (Omar & Rahimtula, 1993). This metabolite is formed by the microsomal fraction of the liver in humans, pigs and rats, in the presence of NADPH to 4(*R*)- and 4(*S*)-4-hydroxy-OTA (Stormer et al., 1981, 1983) via the cytochrome P-450 system (Omar & Rahimtula, 1993; Oster et al., 1991). This metabolite of OTA may be more readily eliminated from the body but may also be as toxic as OTA (Creppy et al., 1983).

OTA is primarily excreted in the urine as OTα and to a lesser degree as OTA. The presence of OTα in urine can be explained by its absorption from the intestine following its formation (Kumagai, 1988). The formation of OTα is the most important mechanism for the removal of OTA, in addition to the excretion of OTA itself (Storen et al., 1982). From our studies, dietary CHA affected the excretion pattern of both OTA and its metabolites (OTα and HO-OTA). Indeed, the presence of CHA in the diet decreased the urinary secretion of OTA and its metabolites, which may be explained by the findings of lower OTA levels in plasma. This is more evident with the diet containing 3 ppm OTA, where excretion rates were higher than with 1 ppm OTA. OTA was also primarily excreted in the urine as OTα and, to a lesser degree, as OTA and HO-OTA, which is in agreement with the study of Storen et al. (1982).

In both bile and feces, OTA was primarily excreted as the intact form and in much smaller amounts as OTα and HO-OTA. The addition of CHA decreased the secretion of OTA and its metabolites in bile. Recent studies have shown that a considerable portion of OTA was secreted in the bile in conjugated forms (glucuronides and sulfates) (Roth et al., 1988), and that the conjugates probably were hydrolyzed to OTA or OTα by the intestinal flora before reabsorption (Delacruz & Bach, 1990). It was also noted that OTA excretion in feces was enhanced by the addition of CHA, while elimination of its metabolites was decreased. Since there was more OTA in feces, it was expected that more OTα would be produced and excreted. However, OTα excretion was lower, which may be attributed to shortened transit time of fecal matter in the intestine (due to presence of

the resin in the diet) and/or decreased exposure of the toxin to bacterial flora (binding of OA by CHA), or even to modification of these flora.

The exact mechanism(s) by which CHA modifies the excretion of OTA in urine and feces is unclear. Alteration of the bile acid pool and enterohepatic circulation of OTA may be one possible mechanism. Bile acids play an important role in the absorption not only of fats and fat-soluble vitamins, but also of xenobiotics (Carey, 1983). It is known that OTA is absorbed in the upper portion of the gastrointestinal tract, mainly in the lipid-soluble nonionized form by diffusional movement (Kumagai, 1988). It is possible that bile acids are required for increase OTA absorption. CHA is an anion-exchange resin used to treat hypercholesterolemia. Administered orally, CHA binds with bile salts in the gastrointestinal tract via ion exchange, breaks their enterohepatic circulation, and increases their fecal elimination (Polli & Amidon, 1995). Thus, it is possible that CHA decreases the OTA bioavailability via a decrease of bile acids required for absorption. Furthermore, preliminary results showed that OTA toxicity was enhanced when rats were fed a phospholipid-enriched diet, known to enhance bile acids secretion (Rioux et al., 1988). This provides further support for the importance of bile acids in OTA absorption, which requires further investigation. Agents that enhance the bile acid secretion should therefore decrease OA toxicity if this mechanism of action is involved in OTA toxicity.

Another explanation for the effects of CHA on OTA toxicokinetics may be binding of the toxin by CHA, which would result in decreased intestinal absorption. When the chemical structure of OA is compared with that of bile acids, there are similarities. Indeed, both molecules contain hydrophobic and hydrophilic domains and have similar molecular weights. Thus, it is hypothesized that these compounds may act similarly in the gastrointestinal tract. OTA has two ionizable groups, the carboxyl group from phenylalanine and the 8-hydroxyl group, both of which can exist in the ionized or nonionized form under physiological conditions. OTA has a $pK_a$ of 7.1 (Chu, 1974) and may exist in its negative form in the lower section of the gastrointestinal tract, where it may with CHA ionically. Under these conditions, the negatively charged OTA molecules would bind to the positively charged groups of CHA. Preliminary results from in vitro studies give support to the view of binding of CHA and OTA (Kerkadi et al., unpublished) as, at least in part, the mechanism by which CHA reduces OTA concentrations in plasma, urine, and bile, and increases its clearance via feces. Resins with more accessible functional groups than CHA may have better capacity to bind OTA and decrease toxicity.

In conclusion, CHA added to the diet reduces the concentrations of OTA in plasma, increases its clearance by way of the feces, and decreases the amount excreted in bile and in urine. By these effects, the kidney is

exposed to lower amounts of the toxin, resulting in decreased renal damage.

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