SHORT COMMUNICATION

Toxicity and Excretion of Ochratoxin A in Rats Intubated with Pure Ochratoxin A or Fed Cultures of Penicillium viridicatum

Toxicity and Excretion of Ochratoxin A in Rats Intubated with Pure Ochratoxin A or Fed Cultures of Penicillium viridicatum. VAN WALBEEK, W., MOODIE, C. A., SCOTT, P. M., HARWIG, J., and GRICE, H. C. (1971). Toxicol. Appl. Pharmacol. 20, 439-441. Rats intubated daily with 500 µg of pure ochratoxin or fed a diet containing 250 µg (daily average) of the toxin present in barley cultures of Penicillium viridicatum Westling developed anorexia and had similar pathological changes in the kidneys. A large part of the intubated ochratoxin A could not be accounted for from analysis of urine and feces, and there was little accumulation of the toxin in the liver or kidneys.

Ochratoxin A is a mycotoxin produced by the molds Aspergillus ochraceus Wilhelm (van der Merwe et al. 1965) and Penicillium viridicatum Westling (van Walbeek et al. 1969). Reports (Krogh and Hasselager, 1968; Cartlon and Tuite, 1970a, b) describing lesions in animals fed grain cultures of P. viridicatum suggest that the toxicity of some of these feeds is due to ochratoxin A. Strains of this fungus are also known to produce the nephrotoxins citrinin and oxalic acid (Scott et al. 1970; Krogh et al., 1970). These observations led us to investigate whether the lesions that develop in rats intubated with pure ochratoxin A resemble those that develop in rats fed barley cultures of an ochratoxin A-producing strain of P. viridicatum. The accumulation of the toxin in liver and kidneys and its excretion were also studied.

Barley cultures B-I and B-II of toxigenic and nontoxigenic strains of P. viridicatum (van Walbeek et al., 1969) were dried, ground, and supplemented with 5% corn oil. Nonmoldy barley (NMB) was prepared in a similar manner. B-I contained 125 ppm ochratoxin A and 1–2 ppm ochratoxin B on analysis (Scott et al., 1970); citrinin (Scott et al., 1970) and oxalic acid (Moir, 1953) were not detected. B-II and NMB contained no ochratoxin A. For 6 days, groups of 10 Wistar male rats (83–110 g) were either intubated daily with 500 µg ochratoxin A (benzene-free) in 0.5 ml 0.1 M sodium bicarbonate and fed NMB (NMB + T), or fed B-I or B-II diets. Control groups were fed NMB ad libitum or in amounts restricted to simulate reduced feed intake by the B-I and NMB + T groups. Thin-layer chromatography (Scott et al., 1970) was used to detect ochratoxin A and the fluorescent metabolite ochratoxin α (Nel and Purchase, 1968) in 24-hr samples of urine (rats fed NMB + T), collected under toluene. Methanol-water (55 : 45, v/v) extracts of feces (daily specimens, rats fed NMB + T) and chloroform-methanol (4 : 1, v/v) extracts of liver and kidney (day 5) were similarly analyzed for these compounds. Routine histologic methods were used for examination of tissues of all animals necropsied on days 3, 5, and 6.

The average total amount of ochratoxin A excreted daily, commencing day 2, in
urine and feces was just over 10% of that intubated (Table 1). Part of the ochratoxin A was metabolized to the hydrolysis product ochratoxin α (Nel and Purchase, 1968). It was not formed in vitro from ochratoxin A, which was stable in rat urine for at least 3 wk at 5°C. The highest amount found in a urine specimen was approximately 27 μg. Small amounts of ochratoxin α (up to 10 μg/rat/day) were also present in the feces. Ochratoxins A and α were observed in urine from rats on diet B-I, but not in urine or feces from control rats.

### TABLE 1

**OCHRATOXIN A IN URINE AND FECES OF RATS INTUBATED DAILY WITH 500 μg OCHRATOXIN A (DIET NMB + T)**

<table>
<thead>
<tr>
<th>Day</th>
<th>Number of rats</th>
<th>Toxin&lt;sup&gt;a&lt;/sup&gt; in urine (μg/rat/day)</th>
<th>Toxin&lt;sup&gt;a&lt;/sup&gt; in feces (μg/rat/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>9</td>
<td>4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.3 ± 1.5</td>
</tr>
<tr>
<td>2</td>
<td>9</td>
<td>20 ± 4</td>
<td>32 ± 7</td>
</tr>
<tr>
<td>3</td>
<td>9</td>
<td>41 ± 7</td>
<td>16 ± 5</td>
</tr>
<tr>
<td>4</td>
<td>6</td>
<td>33 ± 11</td>
<td>30 ± 11</td>
</tr>
<tr>
<td>5</td>
<td>6</td>
<td>43 ± 9</td>
<td>32 ± 5</td>
</tr>
<tr>
<td>6</td>
<td>5</td>
<td>30 ± 13</td>
<td>21 ± 3&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Mean value ± SE.
<sup>b</sup> Approximately.
<sup>c</sup> Two rats only.

Livers and kidneys from 3 rats fed NMB + T and sacrificed on day 5 contained small amounts of ochratoxin A (mean concentration 2.0 and 6.2 μg/g liver and kidney, respectively). Ochratoxin A was also found in the liver and kidneys of the B-I group but no ochratoxin α was detected in the organs from either group.

After day 1, daily feed intake averaged 4.0 g in the NMB + T group and 2.0 g in the B-I group compared to 14.4 g and 13.7 g for the B-II and NMB groups, respectively. Although the barley and corn oil diet may have been nutritionally deficient during this short-term experiment, this was not reflected in the histopathology of rats fed the NMB, restricted NMB, or B-II diets.

Significant histopathologic abnormalities in rats fed B-I or intubated with ochratoxin A were confined to renal tubules. In the kidneys of rats fed NMB + T there was cloudy swelling of proximal convoluted tubular epithelium after 3 days. Rats killed on day 5 had small foci of epithelial vacuolation and cytolysis in the proximal tubules, whereas those killed on day 6 had a bandlike area of tubular epithelial cytolysis and desquamation across the mid-cortex. Hyaline casts were present in some of the kidneys on days 5 and 6. Similar changes were observed in the rats fed B-I diet, but they were not as marked in severity or extent, nor were casts observed in these rats. No renal abnormalities were observed in rats fed NMB or B-II diets.

Comparable kidney lesions have been described (Purchase and Theron, 1968) in rats intubated with single high doses of ochratoxin A (25–40 mg/kg); lower doses produced dilatation of the renal tubules between 4 and 10 days after dosing.
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REFERENCES


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