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Ochratoxin A: Occurrence as Natural Contaminant of a Corn Sample

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Ochratoxin A was detected as a natural contaminant for the first time. It was present in a corn sample.

Ochratoxin A is a toxic metabolite produced by *Aspergillus ochraceus* Wilh., a mold that is widely distributed in nature. South African workers produced feeds toxic to rats, ducklings, and weaned white mice by growing strains of *A. ochraceus* on corn meal, wheat, rye, grain sorghum, rice, buckwheat, soybeans, and peanuts (6). They isolated pure ochratoxin A along with ochratoxins B and C, the respective dechloro and ethyl ester of A, from corn meal inoculated with *A. ochraceus* and incubated (5). Structures of these toxins have been proved by synthesis (3). Van der Merwe et al. (6) found that the acute toxicity of ochratoxin A to ducklings was of the same order as that of aflatoxin B_1. When the toxin was administered orally to 1-day-old ducklings, there was a mild fatty infiltration of the liver; however, comparable doses in weanling rats caused severe liver lesions (4).

There has been no report of any ochratoxin occurring as a natural contaminant in spite of the wide distribution of *A. ochraceus* in nature and the ability of the mold to produce the toxin on a number of substrates.

We decided to analyze for ochratoxin and zearalenone, as well as for aflatoxins, as part of a survey of corn received from commercial markets during 1967. Procedures have been developed whereby the three toxins could be determined simultaneously (2). Samples (50 g) were extracted by shaking with chloroform (250 ml), water (25 ml), and diatomaceous earth (25 g). The mixture was filtered, and hexane (150 ml) was added to a sample (50 ml) of the filtrate. The chloroform-hexane solution was placed on a column (22 mm diameter) packed with anhydrous sodium sulfate (5 g), silica gel (0.05 to 0.2 mm; 10 g), and anhydrous sodium sulfate (15 g). The column was washed with benzene (150 ml), and zearalenone, if present, was eluted with acetone-benzene (5:95, v/v; 250 ml). The column was then washed with anhydrous ether and eluted with methanol-chloroform (3:97, v/v; 150 ml) to remove aflatoxins, if present. Ochratoxin was eluted last with glacial acetic acid-benzene (1:9, v/v; 250 ml). Eluates were concentrated to dryness and taken up in benzene for thin-layer chromatography on silica gel plates (Adsorbosil-1, The Anspec Co., Ann Arbor, Mich.).

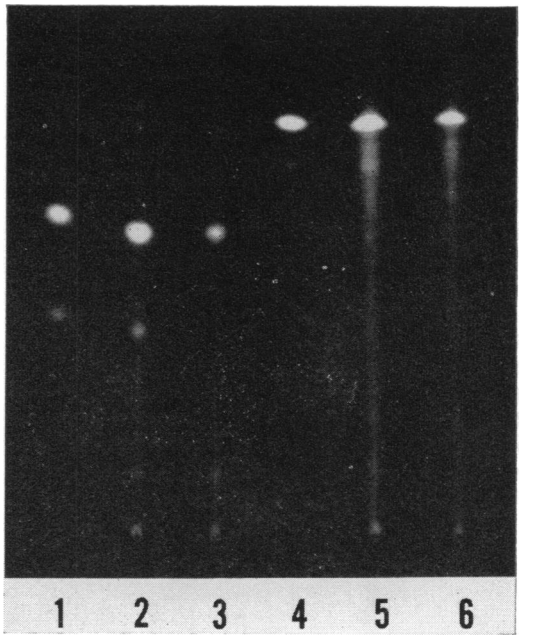

FIG. 1. *Thin-layer chromatogram of ochratoxin A standard and unknown from corn sample F-3972. Standard and its methyl ester were applied at spots 1 and 4, respectively. Unknown and its methyl ester were applied at spots 3 and 6, respectively. Admixtures of unknown and standard and methyl esters were applied at spots 2 and 5. The solvent system was glacial acetic acid-benzene-water (10:90:1, v/v).*

Eluates from one corn sample, F-3972, had a

fluorescing substance with the same mobility on thin-layer plates as ochratoxin A in three solvent systems: glacial acetic acid-benzene-water (10:90:1, v/v), methanol-glacial acetic acid-benzene (5:5:90, v/v; *personal communication*), and toluene-ethyl acetate-90% formic acid (5:4:1, v/v; 2). Zones on silica gel plates containing ochratoxin fluoresce green and turn blue when exposed to ammonia fumes. The same property was exhibited by the compound extracted from corn. Further evidence that the substance could be ochratoxin A was its solubility in 0.1 M sodium bicarbonate (2). The identification of the substance as ochratoxin A was confirmed by the preparation of its methyl ester (*personal communication*). A chloroform solution containing the substance was treated with boron trifluoride (14%) in methanol. When the resulting methyl ester was compared on thin-layer plates with the methyl ester of known ochratoxin A, the two were identical. The solvent system was glacial acetic acid-benzene-water (10:90:1, v/v).

When ground corn sample F-3972 was examined several times for the presence of molds, culture plates had a high incidence of penicillia, a few colonies of *Fusarium*, but no *A. ochraceus*. If *A. ochraceus* was present, it could have become nonviable, perhaps by drying out, but with the toxin level remaining the same. Another possibility is that the mold was not able to produce conidia in the presence of so many penicillia.

Three different portions of the ground suspect corn were extracted to ascertain that the contamination was uniform. Ochratoxin A occurred at levels of 110 to 150 parts per billion. The significance of these levels is not known because the toxicity of ochratoxin has not been thoroughly investigated. Partially purified extracts of the sample inhibited growth of *Bacillus megaterium* NRRL 1368 in a microbiological assay (1) at levels indicating 150 pbp ochratoxin. The assay has been shown to be sensitive to ochratoxin A (N. L. Clements, Abstracts, AACC-AOCS Joint meeting, Washington, D.C., 31 March–4 April 1968). The positive corn was classified as Sample Grade (SG), the poorest grade, and had 18.1% moisture, 1.8% foreign material, 23.3% total damage, and a musty odor. Out of 164 corn samples assayed, only two others (one in Grade 4, the other in Grade 5) contained fluorescing compounds with the same mobility as ochratoxin A on thin-layer plates, but these substances were not soluble in 0.1 M sodium bicarbonate. Included in the 164 were 50 samples in SG, 28 in Grade 5, 37 in Grade 4, 40 in Grade 3, and 9 in Grade 2. Because of the discovery of ochratoxin A as a natural contaminant of corn, even though it may be rare, feeding experiments should be made to determine its effect on farm animals.

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LITERATURE CITED

1. Clements, N. L. 1968. Note on a microbiological assay for aflatoxin B_1: a rapid confirmatory test by effects on growth of *Bacillus megaterium*. J. Ass. Offic. Anal. Chem. **51**:611–612.
2. Eppley, R. M. 1968. Screening method for zearalenone, aflatoxin, and ochratoxin. J. Ass. Offic. Anal. Chem. **51**:74–78.
3. Steyn, P. S., and C. W. Holzapfel. 1967. The synthesis of ochratoxins A and B, metabolites of *Aspergillus ochraceus* Wilh. Tetrahedron **23**:4449–4461.
4. Theron, J. J., K. J. van der Merwe, N. Liebenberg, H. J. B. Joubert, and W. Nel. 1966. Acute liver injury in ducklings and rats as a result of ochratoxin poisoning. J. Pathol. Bacteriol. **91**:521–529.
5. Van der Merwe, K. J., P. S. Steyn, and L. Fourie. 1965. Mycotoxins. II. The constitution of ochratoxins A, B, and C, metabolites of *Aspergillus ochraceus* Wilh. J. Chem. Soc. **1965**:7083–7088.
6. Van der Merwe, K. J., P. S. Steyn, L. Fourie, DeB. Scott, and J. J. Theron. 1965. Ochratoxin A, a toxic metabolite produced by *Aspergillus ochraceus* Wilh. Nature **205**:1112–1113.