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Sensitivity of Aflatoxin B_1 to Ionizing Radiation

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Aflatoxin B_1 in a 5-μg/ml water solution was sensitive to ionizing radiation. Inactivation was assayed by the Ames microsome mutagenicity test and confirmed by thin-layer chromatography. Destruction of aflatoxin B_1 had already begun at 2.5 kilograys (kGy; 1 kGy = 0.1 Mrad), but a dose exceeding 10 kGy was necessary for total destruction.

The stability of aflatoxin with respect to different physical and chemical agents is well-known (6). Autoclaving at 120°C for up to 1 h does not destroy this mycotoxin, and even after sterilization in an acid or alkaline medium, slight mutagenic activity is still detectable (5). In vivo, the activity of aflatoxin B_1 is markedly reduced by hydroxylation at the 9α position. The stability of this mycotoxin is, however, limited in highly polar solvents. Visible light and UV light are also able to stop biological activity (6), but these sources of radiation have a low penetration capacity in solids and liquids. Therefore, the action of gamma rays on aflatoxin B_1 stability was tested.

MATERIALS AND METHODS

Products. Aflatoxins B_1 and G_2 were obtained from Serva Co. (Heidelberg, West Germany), and aflatoxins M_1 and M_2 were obtained from Sigma Chemical Co. (St. Louis, Mo.). The solid products (aflatoxins B_1 and G_2) were solubilized in dimethyl sulfoxide and further diluted in water as needed. Aflatoxins M_1 and M_2 are delivered in chloroform solutions.

Irradiation method. Irradiation was carried out in a Gammator M34-3 irradiation unit (Radiation Machinery Co.). The irradiation source was ^{137}Cs (1,200 Ci). Irradiation was performed at room temperature under air in sealed glass vials at a dose rate of 0.8 kilograys (kGy)/h (1 kGy = 0.1 Mrad).

Biological activity test. Aflatoxin B_1 was tested by the Ames microsome mutagenicity test with *Salmonella typhimurium* TA 98 as a tester strain (2, 4). The base layer was composed of the minimal medium of Vogel (6). The upper layer consisted of four fractions as earlier described (5).

Thin-layer chromatography. After irradiation, the liquids were examined on silica gel 60-coated plates and aluminum oxide-coated plates (F 254 type E; E. Merck A. G., Darmstadt, West Germany) in a 90% $CHCl_3$–10% methanol system. For visualization of the spots, UV light at 366 nm (Minuvis; Desaga, Heidelberg, West Germany) was used. A 0.15-μg amount of aflatoxin B_1 was applied.

RESULTS

Biological assay after irradiation. Table 1 summarizes the biological assay of aflatoxin in a water solution of 5 μg/ml after being exposed to different doses of gamma rays and shows that complete destruction occurred at doses exceeding 10 kGy.

To obtain a quantitative estimate of the degradation rate, the number of revertants was plotted on an aflatoxin B_1 dose-response curve. The quantity of the mycotoxin still present after the different irradiation doses could be estimated at 66, 51.3, 26, and 0% of the original activity with respective doses of 2.5, 5, 10, and 20 kGy. The effect of irradiation was not always the same. In another trial, using only 10 kGy, the undestroyed part was only 10%.

We irradiated a suspension in water of 250 μg of aflatoxin B_1 per ml; the effect of gamma rays was lowered. In this case, 86% was not destroyed with 10 kGy.

Assay on thin-layer chromatography plates of irradiated aflatoxin B_1. The effect of destruction of aflatoxin B_1 was clearly visible on thin-layer chromatography plates. Figure 1 illustrates the chromatogram obtained from silica gel-coated plates. Aflatoxin B_1 was broken down into unidentified components which were still fluorescent. A component with an R_f of 0.38, also present in commercial aflatoxin B_1, resisted 15 kGy but disappeared at 20 kGy. Three different products are clearly seen on the chromatogram of 5-kGy-irradiated aflatoxin B_1 (not including the spot remaining on the spot line). One may suppose that still more fragments would be detected if more sensitive methods were used, such as high-pressure liquid chromatography.

Even with 20 kGy, some traces of aflatoxin B_1 were visible. However, this quantity of undestroyed aflatoxin B_1 falls below the detection limit of the mutagen test, since the Ames test

TABLE 1. Sensitivity of aflatoxin B_1 to gamma irradiation of ^{137}Cs assayed by the Ames microsome mutagenicity test with *S. typhimurium* TA 98

Amt (μg) of aflatoxin B_1 in upper layer before radiation	Revertant colonies[a]/plate with irradiation dose of:				
	2.5 kGy	5 kGy	10 kGy	20 kGy	0 kGy
0.15	1,057	647	215	<1	1,332
0.3	1,169	753	260	<1	1,437
0.6	1,367	833	722	4	1,402

[a] Number of colonies minus the 23 natural revertants.

was negative with an original dosage of 0.6 μg in the upper layer.

When aluminum oxide-coated plates were used, the destruction products remained at the start line. Pure aflatoxin showed no fluorescence on the start line.

Assayed on aluminum oxide, the components appearing after irradiation were different from aflatoxins G_2, M_1, and M_2. Aflatoxin G_2 showed no fluorescence with a 0.15-μg spot, and aflatoxins M_1 and M_2 had very distinct R_f values. The difference with these factors was confirmed on silica gel-coated plates.

DISCUSSION

Aflatoxin contamination in foodstuffs may range from levels of 0.003 (maize) up to 8.6 (peanuts) μg/g, as has been reported. In animal feeds, levels of 0.01 to 0.7 μg/g have been detected (3). These amounts are, in most cases, still lower than the one used in this study (solutions containing 5 μg/ml). It can be expected that aflatoxin B_1 will be destroyed in these materials with an irradiation dose of 10 kGy (the maximum dose presently allowed for irradiated foodstuffs).

However, as is the case for other organic compounds, it is most probable that the physicochemical state of the mycotoxin determines its sensitivity to ionizing radiation. When aflatoxin B_1 was present in our experiments as a precipitate, the effect of irradiation was markedly reduced.

At present, no practical method exists for removing aflatoxin from foodstuffs for human consumption. For animal feed, ammonia vapors at high temperatures have been used for destruction of aflatoxin (A. Garlon, Abstr. Annu. Meet. Assoc. Off. Anal. Chem. 1979, p. 44), and extraction by means of methoxymethane is also efficient for this purpose (1), but both methods are expensive. Therefore, gamma irradiation may be more attractive for mycotoxin decontamination of feed and foodstuffs.

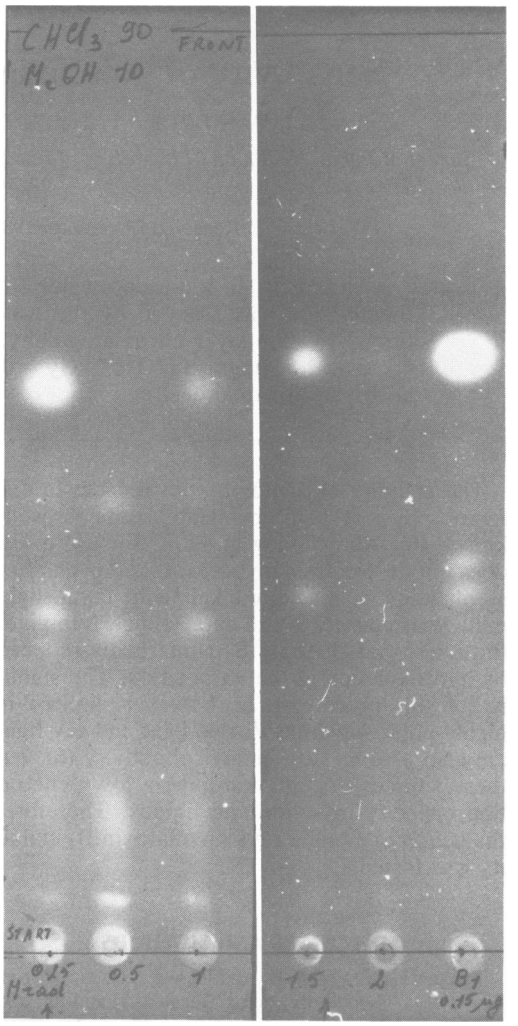

FIG. 1. Thin-layer chromatography of aflatoxin B_1 (5 μg/ml in water) irradiated at five increasing doses as seen under UV of 366 nm, using silica gel-coated plates (20 by 5 cm) and 0.15-μg spots. The control spot of aflatoxin B_1 also shows two other components. Destruction products are most striking at 2.5 and 5 kGy. Nearly all aflatoxin B_1 and its components have disappeared with 20 kGy; a slight fluorescent zone remains on the start line. (Picture on Kodak technical Pan film with Kodak filter 2 E.)

Since only the Ames test was used for demonstrating the disappearance of biological activity, we have only shown that the destruction products are no longer active in bacterial cells; whether this is also the case in mammalian cells remains to be proven. Only in vivo testing can demonstrate complete safety.

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