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Inhibition of Aflatoxin Synthesis by *p*-Aminobenzoic Acid, Potassium Sulfite, and Potassium Fluoride

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Attention has been focused recently on the aflatoxins that are carcinogenic metabolites produced by the *Aspergillus flavus-oryzae* group of fungi (G. N. Wogan, Bacteriol. Rev. **30**:460, 1966). Barium reportedly inhibits aflatoxin synthesis (E. G. H. Lee, P. M. Townsley, and C. C. Walden, J. Food Sci. **31**:432, 1966). There have been no other reports published to date on chemicals that extensively inhibit aflatoxin production per se.

Aspergillus parasiticus Speare var. *globosum* Murikami (*A. flavus* ATCC 15517) was used in these investigations. The organism was cultured in 25 ml of 2% yeast extract and 20% sucrose medium (YES) in 125-ml Erlenmeyer flasks. Flasks of medium were inoculated from spore suspensions and incubated at 27 ± 1 C as stationary cultures for 6 days. Each treatment was replicated four times and aflatoxin analyses were performed on duplicate 1-ml samples of medium that were extracted with two 25-ml portions of chloroform in a separatory funnel. Aflatoxins were determined by thin-layer chromatography (W. A. Pons, Jr., and L. A. Goldblatt, J. Am. Oil Chemists' Soc. **42**:471, 1965). Results of analyses are reported as averages. All figures given are based on 25 ml of medium per flask.

TABLE 1. *Influence of p-aminobenzoic acid (PABA) on growth and aflatoxin production in yeast extract-sucrose medium*

PABA (mg/flask)	Mycelium dry wt (g/flask)	Aflatoxins[a] (mg/flask)
0	0.9	1.20
25	1.0	1.02
50	1.0	0.93
100	1.1	0.69
200	0.6	0.08
300	0.2	0.01
500	0.1	None

[a] Approximately 50% aflatoxin B_1 and 50% aflatoxin G_1.

TABLE 2. *Influence of potassium sulfite on growth and aflatoxin production in yeast extract-sucrose medium*

K_2SO_3 (mg/flask)	Mycelium dry wt (g/flask)	Aflatoxins[a] (mg/flask)
0	0.8	1.05
1	0.7	0.79
10	0.7	0.79
100	0.8	0.61
250	0.8	0.38
500	0.7	0.04
1,000	1.0	0.01

[a] Approximately 50% aflatoxin B_1 and 50% aflatoxin G_1.

p-Aminobenzoic acid (PABA) inhibited aflatoxin production at all concentrations used, and, at the higher concentrations, inhibited growth as well (Table 1). Similar results were obtained with sulfanilamide and anthranilic acid (*data not presented*). Also, aflatoxin elaboration was reduced up to 50% in peanuts that had been soaked in PABA solutions and up to 30% in peanuts sprayed with PABA solution (*data not presented*). Inhibition of aflatoxin synthesis by PABA might be explained as competitive or feedback inhibition on one of the enzymes involved in aflatoxin biosynthesis. There is some evidence that at least a part of the aflatoxin molecule is synthesized via the shikimic acid-aromatic amino acid pathway

TABLE 3. *Influence of potassium fluoride on growth and aflatoxin production in yeast extract-sucrose medium*

KF (mg/flask)	Mycelium dry wt (g/flask)	Aflatoxins[a] (mg/flask)
0	1.0	1.41
1	0.9	1.06
10	0.9	0.94
100	0.9	0.96
1,000	0.4	0

[a] Approximately 50% aflatoxin B_1 and 50% aflatoxin G_1.

through which PABA is synthesized (J. C. Adye and R. I. Mateles, Biochim. Biophys. Acta **86:** 418, 1964).

Potassium sulfite inhibited aflatoxin production without measurably inhibiting the growth of the fungus (Table 2). All concentrations used inhibited aflatoxin production to some extent, whereas sulfite gave virtually complete inhibition at 1% or more. No inhibition was observed with comparable quantities of bisulfite or acetate (*data not presented*). Sulfite is known to inhibit a number of metabolic pathways of fungi, including that of ethyl alcohol production by yeast, in which case it acts as an aldehyde-trapping agent.

Potassium fluoride, particularly at the highest concentration used, also inhibited aflatoxin production and growth (Table 3). Fluoride is known to inhibit enolase activity during glycolysis as well as enzymes of several other metabolic pathways of fungi. The action of fluoride on aflatoxin synthesis supports the contention that at least a part of the aflatoxin molecule is derived via glycolysis. It is known that ^{14}C acetate can be used to label the aflatoxin molecule (J. C. Adye and R. I. Mateles, Biochim. Biophys. Acta **86:** 418, 1964).

Because of their capacity to inhibit aflatoxin production, these compounds warrant further investigation. It would be of interest to know if sulfite or fluoride, when added to the soil or to harvested unshelled peanuts, would prevent post-harvest elaboration of aflatoxin in the kernels. Since PABA is a naturally occurring vitamin, it would be of value to know if it is present in domestic or wild varieties of peanuts and if it may impart a measure of protection against aflatoxin elaboration. Such information might be useful in a peanut-breeding program.

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