Wogan, G. N., Ed., MIT Press, Cambridge, MA, 1964, pp 77–88.
Kinoshita, F., Renbutsu, Y., Khan, J. D., Kohmoto, K., Nishimura, S., *Nippon Shokubutsu Byori Gakkaiho* **38**, 397 (1972).
Lucas, G. B., *Tob. Sci.* **15**, 37 (1971).
McCollough, L. P., Marketing Research Report 518, USDA, Agricultural Marketing Service, 1962.
Meronuck, R. A., Steele, J. A., Mirocha, C. J., Christensen, C. M., *Appl. Microbiol.* **23**, 613 (1972).
Mikami, Y., Nishijima, Y., Iimura, H., Suzuki, A., Tamura, S., *Agric. Biol. Chem.* **35**, 611 (1971).
Pero, R. W., Main, C. E., *Phytopathology* **60**, 1570 (1970).
Pero, R. W., Owens, R. G., Harvan, B., *Anal. Biochem.* **43**, 80 (1971).
Pero, R. W., Posner, H., Blois, M., Harvan, D., Spalding, J. W., *EHP, Environ. Health Perspect.*, 87 (June 1973).
Rosett, F., Sankhals, R. H., Stickings, C. E., Taylor, M. E. U., Thomas, R., *Biochem. J.* **67**, 390 (1957).
Sauer, D. B., Seitz, L. M., Burroughs, R., Mohr, H. E., West, J. L., Milleret, R. J., Anthony, H. D., *J. Agric. Food Chem.* **26**, 1380 (1978).
Schroeder, H. W., Cole, R. J., *J. Agric. Food Chem.* **25**, 204 (1977).
Scott, P. M., Kanhere, S. R., *Abstr., Annu. Meet. Assoc. Off. Anal. Chem.* **93**, 73 (1979).
Slifkin, M. K., Ottolenghi, A., Brown, H., *Mycopathol. Mycol. Appl.* **50**, 241 (1973).
Steyn, P. S., Rabie, C. J., *Phytochemistry* **15**, 1977 (1976).
Umetsu, N., Kaji, J., Tamari, K., *Agric. Biol. Chem.* **37**, 451 (1973).

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Uptake and Metabolism of Aflatoxin by *Zea mays*

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Ten–twelve-day-old maize seedlings were grown for 7 days in Hoagland's solution adulterated with aflatoxin B_1 (AFB_1) plus uniformly ring-labeled [^{14}C]AFB_1. Seedlings were transferred to aflatoxin-free Hoagland's solution or soil to determine the concentration of toxin absorbed and retained within the tissue. Two days following the transfer there was a 75 and 50% reduction in concentration of AFB_1 in the root and leaf–stem tissue, respectively. The reduction may reflect toxin degradation, particularly in the leaf–stem tissue. After 4 days the concentration of toxin increased slightly, suggesting that when the seedlings were transferred to Hoagland's solution there was a desorption of toxin from the root tissue, followed by a reabsorption. Where labeled seedlings were transferred to soil for a period of 13 days and analyzed for aflatoxin there was an 80 and 86% reduction in the concentration of AFB_1 in the root and leaf–stem tissue, respectively. AFB_1 injected into the internode below the ear bearing node in maize showed that it was translocated to developing ears by recovery 33 days later.

Following the discovery that aflatoxins were synthesized by certain strains of *Aspergillus flavus* and *Aspergillus parasiticus*, it was generally assumed that the principal aflatoxin hazard occurred as a consequence of improper field-curing or storage of agricultural commodities, such as corn, peanuts, and cottonseed. However, it is now recognized that *A. flavus* and *A. parasiticus* can infect preharvested standing crops (Lillehoj and Zuber, 1975; Widstrom, 1979). In 1977, the corn crop in southeastern United States was so severely contaminated with aflatoxin caused by *A. flavus* infection that many fields were destroyed by incorporating the stover and grains back into the soil (McMillian et al., 1978). Furthermore, aflatoxin contaminated commodities have been from time to time returned to agricultural soils (Dean, 1979). If aflatoxin is not rapidly degraded by the soil microflora, it is possible that substantial amounts of toxin may be adsorbed by the root systems of subsequent crops and translocated to the foliage and developing fruits. Accumulation of aflatoxin by plants could not only represent a health hazard to the consumer, but may also seriously affect the growth, development, and productivity of plants. This investigation was undertaken to determine if aflatoxin can be absorbed by the root system of plant seedlings, and whether aflatoxin introduced into the stems of corn plants can be translocated to developing fruits.

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MATERIALS AND METHODS

Growth Conditions. Seeds from the maize (*Zea mays*) single cross Mo17 × FRN 28 were soaked in aerated distilled water for 8–12 h. The seeds were rolled in sterile filter paper and germinated at 28 °C for 3–4 days. The seedlings were transferred to large glass tubes (4 × 20 cm) and grown in aerated Hoagland's solution (Hoagland and Arnon, 1950). Following a 7-day growth period uniform seedlings were selected and transferred to Hoagland's solution containing aflatoxin B_1 (AFB_1) plus uniformly ring-labeled [^{14}C]AFB_1 obtained from Moravek Biochemicals (City of Industry, CA). Aflatoxin was introduced into the growth media dissolved in 50 μL of methanol. Following the uptake period the root system was rinsed in tap water, and seedlings were transferred to unlabeled Hoagland's solution or potting soil (peatmoss, perlite, and loam, 1:1:1) and grown for various periods of time before being extracted for the toxin. Three seedlings were extracted for each treatment. Plants were grown in a growth chamber at a constant temperature of 23 °C and a photoperiod of 13 h of light. The light intensity was 200 W/m^2 at the plant level. The growth tubes were wrapped in foil to protect the roots and the aflatoxin solution from light.

In a second study, in order to determine if aflatoxin can be translocated to developing fruits, a corn hybrid with a large kernel was obtained from Cornnuts, Inc. The large kernel facilitated the dissection of the kernel into the

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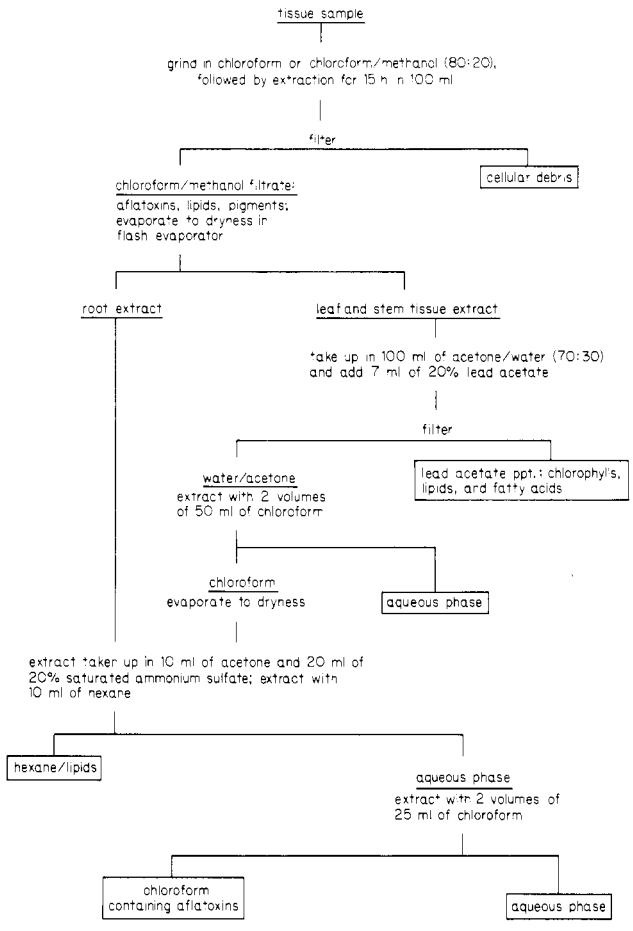

Figure 1. Flow chart for aflatoxin extraction.

component parts of the embryo, pericarp (including aleurone), and endosperm. Fourteen days following pollination a small incision was made in the internode immediately below the developing ear and 10 μL (0.5 μCi) of [^{14}C]AFB$_1$ dissolved in ethanol (180 mCi/mmol) was injected. After treatment, the incision was sealed with silicon grease to prevent the tissue from drying. Thirty-three days later the kernels were harvested and dissected.

Extraction Procedure. The root system was separated from the stem and leaf tissue; however, the stem and leaf tissue were bulked into one component. The tissues were dried for 12 h at 60 °C, weighed, and extracted following the procedure outlined (Figure 1). In order to remove chlorophylls, lipid, and fatty acid contaminants, 20% lead acetate was added to leaf and stem tissue extracts (Hesseltine et al., 1968).

In seedlings that were grown in soil, the final chloroform extract containing aflatoxin was further cleaned up by column chromatography using 10 g of activated silica gel 60–200 mesh according to the procedure outlined in "Official Methods of Analysis" (AOAC, 1975). The chloroform extract (10 mL) was layered on a silica gel column (23 × 290 mm) and eluted first with 150 mL of hexane, followed by 150 mL of ether. The aflatoxin was then recovered in 150 mL of chloroform/methanol (97:3).

Aflatoxin Identification and Quantitation. Authentic AFB$_1$ and unknowns were chromatographed on TLC plates prepared with silica gel G (0.25 mm). The plates were activated for 1 h at 110 °C and developed for 15 cm in unlined tanks by using chloroform/acetone (85:15). The aflatoxin was visualized under long-wave UV, or the TLC plates were scanned by using a Densicord Model 530 densitometer modified for fluorodensitometric analysis according to the method of Pons et al. (1966).

Table I. Effect of pH on the Absorption of AFB$_1$ by Maize Seedlings Grown in Hoagland's Solution[a]

pH	ng of AFB$_1$/g dry weight: root tissue	ng of AFB$_1$/g dry weight: leaf–stem tissue
5.6	9.1	2.7
6.6	17.4	2.4
7.6	20.1	2.9

[a] Four-day-old maize seedlings were transferred to Hoagland's solution containing 0.05 μCi of [^{14}C]AFB$_1$ (sp act., 124 mCi/mmol) at pH 5.6 and 6.6. Because of insufficient isotope only 0.04 μCi was introduced at pH 7.6. The pH was adjusted with 1 M NaOH. After a growth period of 3 days, the seedlings were extracted for aflatoxin.

Confirmation of the identity of AFB$_1$ was made by overspotting with trifluoroacetate (Przybylski, 1975). All procedures were carried out under reduced laboratory light to minimize photochemical changes on TLC plates.

For radioactive determination of [^{14}C]AFB$_1$, samples were counted in a Packard Model 3375 liquid scintillation spectrometer. The scintillation solution contained 5 g of 2,5-diphenyloxazole (PPO), 0.3 g of 1,4-bis[2,4-methyl-5-phenyloxazolyl]benzene (POPOP). Scraping off centimeter sections from TLC plates and suspending the silica gel in thixotropic gel powder revealed that the principal radioactive zone was associated with the R_f value identified as AFB$_1$. The concentration of aflatoxin was therefore calculated on the assumption that the total radioactivity in the various fractions analyzed was AFB$_1$. All data have been correlated for quench and efficiency and are expressed on a per seedling fresh or dry weight basis.

RESULTS AND DISCUSSION

The effect of the hydrogen ion concentration on the uptake of AFB$_1$ by maize seedlings grown for 3 days at various pH values is shown in Table I. The largest concentration of aflatoxin was absorbed by the root system at pH 7.6 with substantial amounts translocated to the leaf and stem tissue. All subsequent uptake studies were conducted at pH 7.6.

Virtually nothing is known about the fate of aflatoxin absorbed by plant tissue; therefore, a study was designed to determine if AFB$_1$ could be metabolized by maize seedlings. Seedlings were grown for 7 days in the presence of [^{14}C]AFB$_1$, followed by transferring to unlabeled Hoagland's solution. Seedlings were harvested every 2 days over a period of 6 days. The data showed that when the seedlings were transferred to unlabeled solution, 1.9 μg of AFB$_1$/seedling was associated with the root tissue and 0.6 μg of AFB$_1$/seedling in the leaf–stem tissue (Table II). Two days after the transfer to an aflatoxin-free solution there was a 75 and 50% decrease in the concentration of AFB$_1$ in the root and leaf–stem tissue, respectively. At 4 days the concentration of aflatoxin increased in both tissues. The initial decline in aflatoxin after 2 days may reflect some degradation, particularly in the leaf–stem tissue; however, the subsequent increase in the concentration of AFB$_1$ suggests that aflatoxin may have been, in part, loosely adsorbed and/or absorbed by the root. Upon transfer of the seedlings to aflatoxin-free media there was desorption, followed by a readsorption. A fluorometric TLC scan of the chloroform extract of root and leaf–stem tissues harvested on the sixth day revealed that there was no other major fluorescing zone evident beyond the R_f value coincident with authentic AFB$_1$ (Figure 2).

Seedlings grown in Hoagland's solution at pH 7.6 tend to become somewhat chlorotic after 5–7 days, probably

Table II. Aflatoxin Recovered during a 6-Day Period from Maize Seedlings Previously Grown in Hoagland's Solution Containing [^{14}C]AFB$_1$[a]

	μg of AFB$_1$/seedling[b]	
days	root tissue	leaf-stem tissue
0[c]	1.9	0.6
2	0.5	0.3
4	0.9	0.8
6	0.8	0.7

[a] Seven-day-old maize seedlings were grown for 7 days at pH 7.6 in 150 mL of Hoagland's solution containing 73.5 μg of stable AFB$_1$ plus 0.23 μCi of [^{14}C]AFB$_1$ (sp act., 180 mCi/mmol). Following the uptake period, the seedling's root system was washed in tap water, and the seedlings were then transferred to AFB$_1$ free medium. Seedlings were removed every 2 days and extracted for AFB$_1$. [b] Average of three seedlings. [c] Uptake at the time of transfer to AFB$_1$ free medium.

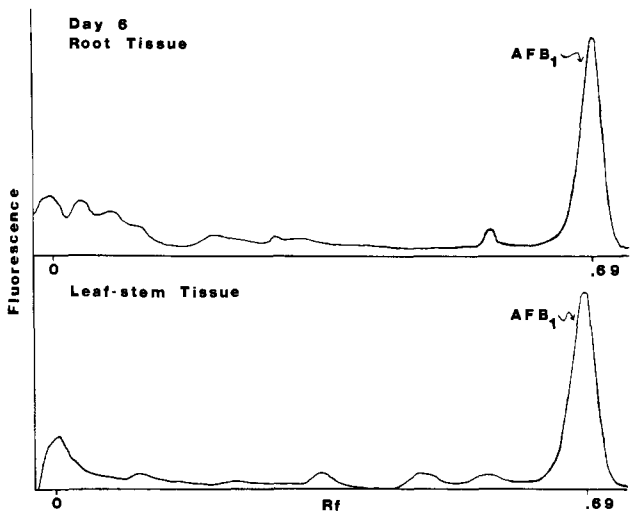

Figure 2. Fluorometric scan of chloroform extract from maize seedlings at day 6.

because of the unavailability of iron at the high pH. In order to alleviate this condition and to extend the growth period over a longer period of time, seedlings were grown for 7 days in radioactive aflatoxin and then transferred to a loam potting soil. Following a 1-week growth period in the soil, the seedlings were harvested every 2 days over a 6-day period and extracted for AFB$_1$. Because of the extended growth period there was roughly a 10-fold increase in dry weight. This increase in tissue caused a large amount of interfering fluorescing material in the chloroform extract that prevented positive identification of AFB$_1$ on the TLC plates. However, a determination of the radioactivity in the chloroform extract revealed that over 13 000 dpm/seedling were associated with both the root and leaf–stem tissue at the time the seedlings were tranferred to the soil (Table III). After 7 days of growth in the soil there was approximately a 70% decrease in radioactivity, followed in turn by an increased accumulation of radioactivity over the next 6 days. The initial decrease in radioactivity in the root tissue suggests a metabolic breakdown of the aflatoxin molecule or a temporary desorption from the root. Subsequent samplings indicated an apparent reabsorption which parallels new root proliferation. However, in the leaf-stem tissue the initial decline in the concentration of aflatoxin suggested a breakdown, followed by reabsorption and translocation of toxin. The recovery of aflatoxin at 0, 7, and 13 days by silica gel chromatography is illustrated in Table IV. After

Table III. Total Radioactivity Recovered in the Chloroform Extract during a 6-Day Period from Maize Seedlings Grown in Soil[a]

	dpm/seedling[b]	
days	root tissue	leaf-stem tissue
0[c]	13189	13837
7	3678	4512
9	7336	6511
11	7214	10003
13	8355	12320

[a] Seven-day-old maize seedlings were grown for 7 days at pH 7.6 in 150 mL of Hoagland's solution containing 73.5 μg of AFB$_1$ plus 0.23 μCi of [^{14}C]AFB$_1$ (sp act., 180 mCi/mmol). Following the uptake period, the root system was rinsed in tap water, and the seedlings were then transferred to potting soil. Following a 7-day growth period in soil, the seedlings were harvested every 2 days and extracted for AFB$_1$. [b] Average of three seedlings. [c] Uptake at the time of transfer to soil.

Table IV. Recovery of AFB$_1$ from Maize Seedlings Grown in Soil[a]

	μg of AFB$_1$/seedling[b]	
days	root tissue	leaf-stem tissue
0[c]	1.5	1.4
7	0.3	0.4
13	0.3	0.2

[a] The chloroform extracts from Table III were purified by silica gel column chromatography. [b] Average of three seedlings. [c] Uptake at time of transfer to soil.

7 days growth in the soil, AFB$_1$ decreased by 80 and 72% in the root and leaf–stem, respectively. After 13 days, AFB$_1$ decreased by 86% in the leaf–stem tissue with no further change in the root tissue. The apparent decrease in the level of AFB$_1$ following column chromatography is in contrast to the data presented in Table II, where there was a small increase in aflatoxin following the second day sampling. It is possible that the fluorescing zones chromatographically identified as AFB$_1$ in Table II possessed increasing concentrations of radioactive breakdown products which masked an actual decrease in aflatoxin. It is also possible that desorbed aflatoxin was not altered by microflora in hydroponic cultures because of the relatively sterile Hoagland's solution; therefore, the slight increase truly represents reabsorbed AFB$_1$. Desorption of AFB$_1$ from seedling root grown in soil could be partially degraded by the soil microflora; therefore, the increased accumulation of radioactivity recorded in Table III could represent the absorption of breakdown products of aflatoxin. Regardless of the interpretation placed on the discrepancies between the two sets of data a decrease in the concentration of AFB$_1$, particularly in the leaf–stem tissue, suggests that the plant was able to metabolically degrade aflatoxin.

In recent years a number of studies reported that aflatoxins have been isolated from apparently sound, intact seeds at all stages of development and maturity (Anderson et al., 1975; Cucullu et al., 1966; Shotwell et al., 1974). If the seed coat was truly intact the recovery of aflatoxin could have resulted from the translocation of the toxin from contaminated soils or from sites of fungal infection and synthesis of the toxin other than in the seed per se. In order to determine if aflatoxin can be translocated to developing fruits 0.5 μCi of [^{14}C]AFB$_1$ dissolved in 10 μL of ethanol was introduced into the stem subtending the developing ear of maize 14 days following pollination.

Table V. AFB_1 Uptake by Developing Seeds of Maize[a]

	ng/g fresh weight
pericarp (aleurone)	18.3
embryo	18.2
endosperm	0.7
total	37.2

[a] Ten microliters (0.5 μCi) of [^{14}C]AFB_1 (sp act., 180 mCi/mmol) was injected through a small incision in the stem subtending the developing ear 14 days following pollination. Thirty-three days later the seeds were dissected into pericarp, embryo, and endosperm. Data represent the total [^{14}C]AFB_1 recovered by chloroform extraction.

Thirty-three days later an equivalent of 18 ng of [^{14}C]-AFB_1/g fresh weight was recovered from the pericarp and embryo with only traces (0.7 ng) in the endosperm (Table V). A radiochromatographic scan with a Packard Model 385 scanner of the chloroform extract revealed a radioactive zone that cochromatographed with authentic AFB_1; however, positive identification by chemical derivatization was not made. The isolation of AFB_1 from the seed illustrates that aflatoxin can be recovered from intact grain in which there was no evidence of fungal contamination. Furthermore, the accumulation of [^{14}C]AFB_1 by the pericarp and embryo may explain why aflatoxin is frequently recovered in the gultin fraction following the wet milling process of grain.

The isolation of toxin from seedlings grown in Hoagland's solution adulterated with AFB_1 and the recovery of toxin from grain following the injection of AFB_1 into the internode subtending the developing ear raise the question whether these processes occur under field conditions. If they do, precautions should be exercised in the disposal of aflatoxin contaminated commodities back into the soil where subsequent crops are to be grown.

The results of this study indicate additional research is urgently needed to establish whether aflatoxin exists in field soils where it can be absorbed by roots. Research is also needed on the effect of different soil types and environment on the degradation of aflatoxin by microflora.

LITERATURE CITED

Anderson, H. W., Nehring, E. W., Wichser, W. R., *J. Agric. Food Chem.* **23**, 775–782 (1975).
Association of Official Analytical Chemists, "Official Methods of Analysis", 12th ed., Washington, DC. 1975, Chapter 26.
Cucullu, A. F., Lee, L. S., Mayne, R. Y., Goldblatt, L. A., *J. Am. Oil Chem. Soc.* **43**, 89–92 (1966).
Dean, G. C., Compliance Officer, Food and Drug Administration, Denver Field Office, personal communication, 1979.
Hesseltine, C. W., Shotwell, O. L, Smith, M. L., Shannon, G. M., Vandergraft, E. E., Goulden, M. L., *Mycologia* **60**, 204–312 (1968).
Hoagland, D. R., Arnon, D. I., *Circ.—Calif. Agric. Expt. Stn.* **347** (1950).
Lillehoj, E. B., Zuber, M. S., *Proc. Annu. Corn Sorghum Res. Conf. 30th*, 230–250 (1975).
McMillian, W. W., Wilson, D. M., Widstrom, N. W., *J. Expt. Qual.* **7**, 564–566 (1978).
Pons, W. A., Jr., Robertson, J. A., Goldblatt, L. A., *J. Am. Oil Chem. Soc.* **43**, 665–669 (1966).
Przybylski, W., *J. Assoc. Off. Anal. Chem.* **38**, 110–113 (1975).
Shotwell, O. L., Goulden, M. L., Hesseltine, C. W., *Cereal Chem.* **51**, 492–499 (1974).
Widstrom, N. W., *J. Expt. Qual.* **8**, 5–11 (1979).

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Effect of Preprocessing Procedures for Green Bellies on *N*-Nitrosopyrrolidine Formation in Bacon

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The effect of pork belly storage on nitrosopyrrolidine (NPYR) formation in fried bacon was investigated. Bacon made from fresh bellies produced significantly less ($p < 0.05$) NPYR than that made from bellies that had been either stored for 1 week in a refrigerator or frozen for 3 months and then thawed prior to use. Bellies thawed in water produced less NPYR than bellies thawed in a refrigerator or at room temperature. A high correlation ($p < 0.01$) between residual nitrite and NPYR was also observed.

Recent research has focused on devising methods for the inhibition of nitrosamine formation in fried bacon, especially *N*-nitrosopyrrolidine (NPYR). Different approaches to solving the problem have been followed, including the determination of the precursor species (Gray, 1976; Nakamura et al., 1976; Gray and Collins, 1978; Gray et al., 1978; Cassens et al. 1979; Bharucha et al., 1979); determination of the variables affecting production of these compounds (Herring, 1973); determination of the cooking methods and the temperatures that reduce nitrosamine formation (Pensabene et al., 1974; Sen et al., 1976a; Mattson, 1978; Wasserman et al., 1978). Attention has also been given to reduction of levels of nitrite in the cure mixture (Sen et al., 1974; Havery et al., 1978) and the addition of water- and lipid-soluble reductants to the cure (Herring, 1973; Mottram et al., 1975; Hwang and Rosen, 1976; Sen et al., 1976b; Walters et al., 1976; Coleman, 1978; Fiddler et al., 1978). In the latter case, inhibition of ni-

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