Also, this is the first report of a fungus outside the genera of *Claviceps* and *Balansia* (Bacon et al., 1979, Porter et al., 1979) that can produce alkaloids that are N-peptide-substituted amides of lysergic acid. Dihydroergosine has been reported in isolates of *Sphacelia sorghi* (Mantle and Waight, 1968). We have no evidence for the in vivo production of the three alkaloids we isolated. The final assessment of toxicity should be made in the light of the systemic habit of *E. typhina*, which suggests that the grass might induce biotransformation of the alkaloids, that environmental influences may affect the alkaloid accumulation, and that the fungus and host interact during the growing season to produce toxic compounds.

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LITERATURE CITED

Agurell, S., *Acta Pharm. Suec.* **2**, 357 (1965).
Bacon, C. W., Porter, J. K., Robbins, J. D., Luttrell, E. S., *Appl. Environ. Micro.* **34**, 576 (1977).
Bacon, C. W., Porter, J. K., Robbins, J. D., *J. Gen. Microbiol.*, in press (1979).
Cassady, J. M., Abou-Chaar, C. I., Floss, H. G., Lloydia, *J. Nat. Prod.* **36**, 390 (1973).
Floss, H. G., *Tetrahedron* **32**, 873 (1976).
Flückiger, E., Doepfner, W., Marko, M., Niederer, W., *Postgrad. Med. J.* **52**, 57 (1976).
Haley, T. J., Andem, M. R., Liebig, C., *Arch. Int. Pharmacodyn.* **98**, 373 (1954).
Hofmann, A., "Die Mutterkornalkaloide", Enke Verlag, Stuttgart, 1964.
Jacobson, D. R., Hatton, R. H., Proceedings of Fescue Toxicity Conference, Lexington, KY, May 31–June 1, 1973, p 4.
Loew, D. M., "Ergot Alkaloids and Related Compounds", Vol. 49, Berde, B., Schield, H. O., Ed., Springer-Verlag, New York, 1978, p 455.
Maag, D. D., Tobiska, J. W., *Am. J. Vet. Res.* **17**, 202 (1956).
Mantle, P. G., Waight, E. S., *Nature (London)* **218**, 581 (1968).
Porter, J. K., Bacon, C. W., Robbins, J. D., *J. Agric. Food Chem.* **22**, 838 (1974).
Porter, J. K., Bacon, C. W., Robbins, J. D., Lloydia, *J. Nat. Prod.*, in press (1979).
Saameli, K., "Ergot Alkaloids and Related Compounds", Vol. 49, Berde, B., Schield, H. O., Ed., Springer Verlag, New York, 1978, p 233.
Simpson, H. B., *N.Z. Vet. J.* **23**, 182 (1975).
Smith, S., Timmis, G. M., *J. Chem. Soc.*, 396 (1937).
Stahl, E., "Thin-Layer Chromatography, a Laboratory Handbook", No. 74, Springer-Verlag, New York, 1969, p 869.
Voigt, D., Johne, S., Groger, D., *Pharmazie* **29**, 697 (1974).
Vokoun, J., Sajdl, P., Rehacek, Z., *Zentralbl. Bakt. Abt. Bd.* **129**, 499 (1974).
Vokoun, J., Rehacek, Z., *Collect. Czeck. Chem. Commun.* **40**, 1731 (1975).
White, A. C., *Q. J. Pharm. Pharmacol.* **17**, 1 (1944a).
White, A. C., *Q. J. Pharm. Pharmacol.* **17**, 95 (1944b).
Yates, S. G., "Microbial Toxins", Vol. 7, Kadis, S., Ciegler, A., Ajl, S. J., Ed., Academic Press, New York, 1971, p 191.

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[^{14}C]Aflatoxin B_1 as an Indicator of Toxin Destruction during Ammoniation of Contaminated Peanut Meal

Louise S. Lee,* Edith J. Conkerton, Robert L. Ory, and Joan W. Bennett

[^{14}C]Aflatoxin B_1 was used to trace the destruction of aflatoxin during ammoniation. "Spiked" meal (meal containing added [^{14}C]aflatoxin) was ammoniated at 75 °C, 35 psig, for 30 min in a laboratory reactor, and the distribution of label was measured in subsequent fractions. Only 45–50% of the activity was detected in the ammoniated meal after initial air-drying, 8% was detected in the humins remaining after acid hydrolysis, 4–6% was associated with the protein, and 33–36% was associated with the nonprotein residue. This residue contained all of the unreacted aflatoxin B_1, which accounted for 0.3% of the total activity. Some activity was detected in volatiles swept from the reaction vessel after the ammoniation treatment.

Treatment with ammonia gas at elevated temperatures and pressure (Dollear, 1969) effectively inactivates aflatoxins in oilseed meals. In 1971, Gardner et al. determined that treatment of contaminated cottonseed and peanut meals with ammonia gas at 40–50 psig for 30 min at 95–125 °C reduced the aflatoxin content to less than 1 μg/kg. Since then, Lee et al. (1974) and Cucullu et al. (1976) studied the products formed in a model reaction of aflatoxin B_1 and ammonium hydroxide with heat and pressure. Of the original aflatoxin B_1, 30% was accounted for in this model reaction; 10% of this was converted to a M_r 286 compound (aflatoxin D_1), 10% to a M_r 206 compound, and 10% remained unreacted. In 1978, Lee and Cucullu studied the conversion of aflatoxin B_1 to these products during ammoniation of meals spiked with aflatoxins and in meals prepared from peanuts cultured with *Aspergillus parasiticus*. They report a 0.24% conversion of B_1 to D_1 in meal prepared from cultured peanuts and a 0.16% conversion during ammoniation of a peanut meal spiked with aflatoxin B_1. No M_r 206 compound was detected. After ammoniation of the meal prepared from

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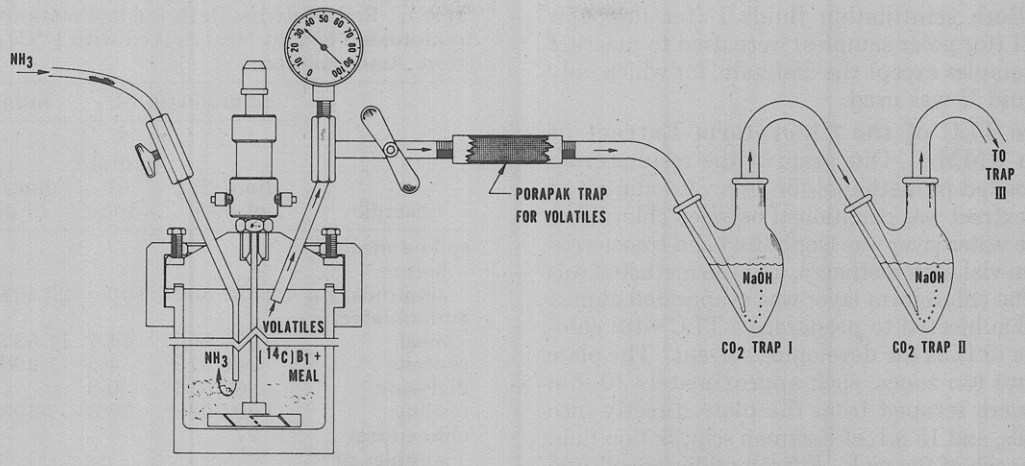

Figure 1. Diagram of the ammoniator showing gas inlet, gas outlet, and the Porapak P and sodium hydroxide traps.

cultured peanuts, unreacted aflatoxin B_1 remaining was 0.18% and was 0.21% after a similar ammoniation of spiked meal. Since products formed during ammoniation were similar for both spiked and cultured samples, only spiked samples were used in the present study. The purpose of these experiments was to determine the fate of the 99% aflatoxin B_1 that was not accounted for as either unreacted B_1 or B_1 converted to D_1 in ammoniated meals. Measurement of radioactivity of various fractions of ammoniated meal spiked with [^{14}C]aflatoxin B_1 prior to ammoniation would determine the extent of association of aflatoxin B_1 or fragments of aflatoxin B_1 with meal components during ammoniation.

EXPERIMENTAL PROCEDURES

Preparation of [^{14}C]Aflatoxin B_1. Radioactive aflatoxin B_1 was prepared by the addition of sodium 1-[^{14}C]acetate to resting cell cultures of *A. parasiticus*. The procedure used by Lee et al. (1976) for the preparation of [^{14}C]aflatoxin B_1 from acetate was followed, except that twenty-five 50-mL flasks were used in each of two sets of B_1 preparation, and 0.1-mL aliquots of acetate (1 mCi/10 mL of water) were added at the beginning and at three 1.5-h intervals. Both the thin-layer chromatographic (TLC) purification and the estimation of radioactivity were identical with procedures described by Lee et al. (1976). An excess of 4 mg of labeled aflatoxin B_1 was obtained from each preparation, presumably labeled according to the pattern reported by Biollaz et al. (1970). Preparations were weighed separately and dissolved in chloroform at a concentration of 4 mg/5 mL. Radioactivity of an aliquot of each preparation was measured on a Searle Isocap/300 liquid scintillation system.

Ammoniation of Peanut Meal Spiked with [^{14}C]-Aflatoxin B_1. Raw, blanched peanuts were sliced, hexane extracted, air-dried, and ground in a Wiley mill to pass a 20-mesh screen. The meal was adjusted to 15% moisture. Four milligrams of [^{14}C]aflatoxin B_1 was added in chloroform solution to each of two 20-g samples of the meal, and the chloroform was evaporated. Each 20-g sample of spiked meal was ammoniated in the Parr reactor used by Lee and Cucullu (1978) under their mildest conditions of ammoniation (30 min, 75 °C, 35 psig ammonia pressure). Temperature was maintained at ±5 °C and pressure at ±5 psig by opening the outlet valve shown in Figure 1 at intervals during ammoniation. Ammoniated meal was removed from the reactor and air-dried. These ammoniations are designated AMM 1 and AMM 2.

Ammoniated Meal Separated by Solubility. Five-gram portions of meal from AMM 1 and AMM 2 were extracted with 10% sodium chloride adjusted to pH 7 (1:10, w/v). The soluble portion was separated from the residue by centrifugation. The method described by Conkerton et al. (1973) was used, except that dialysis to remove excess salt was performed overnight in the apparatus shown in Figure 2.

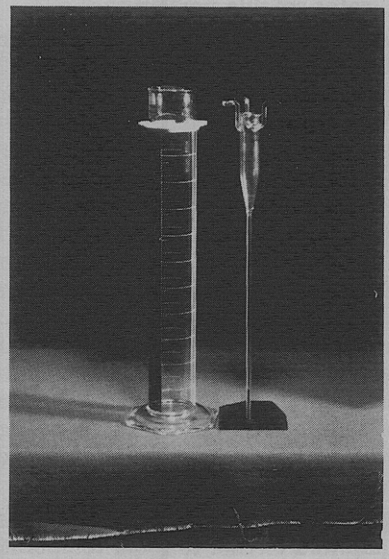

Figure 2. Photograph of the apparatus used to dialyze the protein. A 1-L graduated cylinder supports the glass holder, constructed from a 40-mL glass, conical-tipped centrifuge tube. The tube, opened at the bottom, is connected to a glass tube to allow for water circulation. A dialysis bag (not shown) containing the protein is placed in the conical tube.

This unique, all-glass apparatus allowed the dialysis bag to be continually bathed with a relatively small volume of water. The dialysate, rich in salt, collected in the bottom of the graduated cylinder. The density gradient produced a continuing supply of salt-free water around the dialysis bag.

Dialysate, residue, and salt-soluble protein from AMM 1 were lyophilized separately, and the dried samples were weighed. Only the residue and protein fractions of meal from AMM 2 were lyophilized. The dialysate was discarded.

Radioactivity in each fraction was measured in the following manner: aliquots of the original spiked meals, ammoniated meals, protein, residue from AMM 1 and AMM 2, and the dialysate from AMM 1 were weighed and transferred to 10-mL volumetric flasks. The samples were made to volume with methanol, and 1-mL aliquots were pipetted from each flask into scintillation vials. After evaporation of the methanol, 15 mL of scintillation fluid

was added. Both scintillation fluids I (for nonpolar samples) and II (for polar samples) were used to measure activity of all samples except the dialysate, for which only scintillation fluid II was used.

Preparative TLC of the Chloroform Extract of Residue from AMM 1. One gram of the residue from AMM 1 was soaked in methanol for 24 h. An aliquot of this methanol extract was partitioned between chloroform and water. The water layer was lyophilized and transferred to a scintillation vial with methanol, and the methanol was evaporated. The chloroform layer was evaporated almost to dryness and subjected to preparative TLC with chloroform–acetone (9:1) as the developing solvent. The plate was divided into ten zones, each approximately 10 mm wide. Zones were scraped from the plate directly into scintillation vials, and 15 mL of Eastman scintillation fluid I was added to each of the vials. Fifteen milliliters of fluid II was added to a scintillation vial that contained the lyophilized, water-soluble portion.

Acid Hydrolysis of AMM 1. Ten grams of the meal from AMM 1 was soaked in methanol for 24 h. The methanol extract was decanted, and the procedure was repeated. The meal was then transferred to a fritted funnel, where it was vacuum-washed repeatedly with methanol. An aliquot of the methanol extracted meal was transferred to a scintillation vial, and the remainder was placed in two 250-mL round-bottom flasks fitted with screw caps. Acid hydrolysis was conducted in an atmosphere of nitrogen according to the procedure of Moore and Stein (1963). The hydrolysate and the black insoluble humins were separated, lyophilized, and weighed. An aliquot of each was transferred to a scintillation vial, and 15 mL of scintillation fluid II was added to each vial. A few drops of sodium dodecyl sulfate, a strong detergent used to solubilize difficult samples, was also added to the humins sample.

Trapping Experiments. After AMM 2, the unopened reactor was allowed to cool to room temperature. In an attempt to trap any volatile fragments of [^{14}C]aflatoxin B_1 produced during ammoniation, a series of traps was attached to the outlet of the ammoniator, as shown in Figure 1. The Porapak trap was a glass tube (10 × 100 mm) packed with Porapak P (styrene–divinylbenzene porous polymer), a GLC column packing material. A series of three traps, each filled with 30 mL of 1 N sodium hydroxide, was attached in tandum after the Porapak trap.

Nitrogen was allowed to pass into the reactor through a tube from a tank to the gas inlet and was drawn out through the traps by vacuum. This sweeping action was continued for 6 h. The Porapak trap was removed and the packing material quickly transferred to a scintillation vial. Fifteen milliliters of scintillation fluid II was added to the vial. Contents of each sodium hydroxide trap were transferred to graduated cylinders, and an equal volume of 1 M barium chloride was added to each cylinder. The insoluble carbonate precipitates were collected on small, filter paper disks placed inside coarse-fritted funnels. After washing with water under vacuum, the disks with the precipitates were transferred to scintillation vials, and 15 mL of scintillation fluid II was added. The supernatant alkaline layers were combined and extracted with chloroform, and the extract was concentrated to about 5 mL and transferred to a scintillation vial. The solvent was evaporated almost to dryness, and 15 mL of scintillation fluid I was added.

Measurements of Radioactivity and of Aflatoxins. All samples from the above experiments were counted on the Searle Isocap Scintillation Counter. Disintegrations per minute (dpm) were calculated from counts measured on aliquots of each sample and were corrected from a ^{14}C source quench curve. Weights of aliquots were used to calculate all values as dpm/20 g of the original ammoniated meals.

Table I. Radioactivity Detected in Fractions of Ammoniated Peanut Meal Spiked with [^{14}C]Aflatoxin B_1 before Ammoniation

	ammoniation 1		ammoniation 2	
sample	dpm/20 g of meal	% total of dpm	dpm/20 g of meal	% total of dpm
spiked meal before ammoniation	22 537 500[a]	100	23 468 000[a]	100
ammoniated meal	10 077 900[a]	44.7	11 687 000[a]	49.8
protein	946 575[a]	4.2	1 290 740[a]	5.5
dialysate	67 613[b]	0.3		
residue	7 504 988[a]	33.3	7 603 632[a]	32.4
chloroform solubles of residue	7 459 913[c]	33.1	7 560 658[c]	32.2
water solubles of residue	none[b]		none	
humins	1 606 050[d]	8.0		

[a] Average of duplicate measurements made from methanol extracts of the samples counted in scintillation fluids I and II. Variation: ±6% in fluid I; ±5% in fluid II. [b] Average of duplicate measurements made from methanol extracts of the samples counted in scintillation fluid II. Variation ±5%. [c] Average of duplicate measurements made from aliquots of the chloroform extract counted in scintillation fluid I. Variation ±5%. [d] Single measurement made in scintillation fluid II. Correction for blank made.

Weighed aliquots of the ammoniated meal, protein, residue, and dialysate from AMM 1 and ammoniated meal, protein, and residue from AMM 2 were analyzed chemially for aflatoxin B_1 and D_1. The method of Pons et al. (1968) was used to estimate aflatoxin B_1, and the method of Lee et al. (1978) was used to estimate aflatoxin D_1 formed during ammoniation.

RESULTS AND DISUSSION

Chemical analyses showed that all of the aflatoxin B_1, unchanged by ammoniation, and the aflatoxin D_1, formed during ammoniation, were detected in the original ammoniated meal and in the residue. No aflatoxins were chemically detected in the dialysate or associated with the protein portions of peanut meal. Only 0.2% of the added aflatoxin B_1 was detected as B_1, and a similar amount of aflatoxin D_1 was formed from B_1 during ammoniation. These values for B_1 and D_1 are in good agreement with those reported by Lee et al. (1978) in their study on ammoniated, cultured meals and meals spiked with aflatoxin B_1.

All other results reported in Tables I, II, and III are of radioactive measurements. Radioactivity of aflatoxin B_1, measured on an aliquot of each spiking solution, was 24 637 800 dpm for B_1 added to the 20 g of meal before AMM 1 and 25 722 600 dpm for B_1 added to 20 g of meal before AMM 2. Other results reported in Table I list the radioactivity detected in the meal + B_1 before ammoniation and in various fractions of the ammoniated meals. Results are presented as percent of the total radioactivity in the original 20 g of meal as well as dpm/20 g of meal. For AMM 1 and AMM 2, values obtained on the spiked meals are slightly lower (22 537 500 and 23 468 000 dpm) in measurements made on meal + B_1, rather than on B_1

Table II. Radioactivity Detected in Zones Harvested by Preparative TLC of the Chloroform-Soluble Portion of Meal Residue

	TLC zones[a]	dpm/20 g	% total ^{14}C
1[b]	dark, nonfluorescent	157 763	0.7
2	blue fluorescent	202 838	0.9
3	dark, nonfluorescent	45 075	0.2
4	blue fluorescent; B_1	78 150	0.3
5	light blue, sl. fluorescent	1 983 330	8.8
6	dark, nonfluorescent	2 559 425	11.4
7	gray fluorescent; D_1	127 763	0.6
8	dark, nonfluorescent	292 988	1.3
9	light blue, nonfluorescent	991 650	4.4

[a] Silica gel GH-R plate developed in chloroform–acetone (9:1). Developed plate divided into ten zones, approximately 10 mm each. [b] Solvent front. [c] Origin.

Table III. Radioactivity Detected in Volatiles Trapped after Ammoniation of Peanut Meal Spiked with [^{14}C]Aflatoxin B_1 before Ammoniation

trap	dpm/20 g of meal[a]	% of total dpm
Porapak	19420	0.1
NaOH I	3220	0.1
NaOH II	3596	0.1
NaOH III	3110	0.1
combined supernatants from traps I, II, III	3668	0.1

[a] Meal reported in Table I under ammoniation 2.

alone. These differences are probably due to difficulty in sampling spiked meal.

Distribution of the 45–50% radioactivity remaining after initial air-drying indicates that most of the activity is associated with materials in meal residue rather than with protein. Whereas Natarajan et al. (1975) report that 80% of the aflatoxins of a naturally contaminated peanut sample are fractionated along with the protein during wet milling, our results with meal spiked with B_1 indicate that during ammoniation, the aflatoxin, if associated with the protein, breaks down into fragments that disassociate from the protein and are detected in the residue. Only 4.2–5.5% of the original radioactivity was associated with the protein portion, and just 0.3% was detected in the dialysate. Loss of some sample was encountered during separation and lyophilization. This loss was reflected in the weights of the subfractions and, consequently, in the radioactivity.

Residue after removal of the protein by extraction in 10% salt solution contained 32.4–33.3% of the total activity. This separation was based on solubility, and these radioactive materials, produced during ammoniation of [^{14}C]aflatoxin B_1 in peanut meal, were simply insoluble in the aqueous salt solution.

Results of the acid hydrolysis experiment, however, suggest that at least part of the radioactivity that was not detected in the separation by solubility is bound to cell walls or other large molecular weight particles that would also be part of the residue. These results agree with those of Beckwith et al. (1975), who showed that aflatoxin B_1 is bound to starch molecules in corn ammoniated 30 h with no heat. Acid hydrolysis of methanol-extracted meal should have detached any aflatoxin or aflatoxin fragments chemically bound to the meal during ammoniation. The final methanol wash of the ammoniated meal contained less than 0.5% (112 688 dpm) of the total radioactivity, yet the methanol-extracted ammoniated meal still retained 1 431 000 dpm (6.3%) of the original activity. This measurement was made on an aliquot of the meal placed directly in the scintillation vial. Similar measurements of radioactivity made directly on the humins indicate that 8% of the original activity is associated with this black insoluble residue from acid hydrolysis (Table I). Such materials are different from those accounted for in meal residue in the fractionation by solubility. The fact that the meal contained measureable radioactivity even after exhaustive methanol extraction indicates that some of the [^{14}C]aflatoxin B_1 must be closely bound to meal particles. The material responsible for this activity was not extractable with methanol and was detected in the methanol-extracted meal and in meal humins after acid hydrolysis.

Results of the preparative TLC experiments are reported in Table II. Virtually all of the 33.3% radioactivity detected in the residue (Table I) was partitioned into chloroform. Radioactivity was distributed throughout the developed TLC plate. This indicates that the chloroform-soluble products formed from ammoniation of aflatoxin B_1 are probably fragments of aflatoxin B_1 or are low-molecular-weight compounds. Large-molecular-weight materials formed from strong bonding of B_1 to meal residue would not have moved in the solvent system employed. Only 0.3% of the radioactivity of the original B_1 was detected in zone 4 and 0.6% in zone 7. In this solvent system, aflatoxin B_1 (M_r 312) has an R_f identical with that of the fluorescent material in zone 4, and aflatoxin D_1 (M_r 286) has an R_f identical with that of the material constituting zone 7. These values of 0.3 and 0.6% for B_1 and D_1, respectively, are higher than those obtained by chemical analysis, probably because other radioactive materials were scraped from the plate along with aflatoxins B_1 or D_1. The greatest amount of total activity (20.2%) was contained in zones 5 and 6—zones between aflatoxins B_1 and D_1. By nature of their mobility in this solvent system, materials accounting for this activity are less polar than B_1 and are more polar than D_1. The two nonfluorescent zones at the origin and immediately above it each contain material more polar than either aflatoxin B_1 or D_1. These materials constitute 8.5% of the total radioactivity.

Results of the trapping experiment are reported in Table III. Since only 45–50% of the added label was accounted for in the air-dried, ammoniated meals, it is reasonable to expect that about half of the initial radioactivity would be detected in the volatiles. Actual recovery of radioactivity as volatiles was poor—our technique trapped less than 1% of the total activity. There are several plausible explanations for this. Some radioactive volatiles were probably released when the outlet valve was opened at intervals to maintain the desired pressure throughout the ammoniation process. Moreover, the Porapak P was swamped with ammonia gas, which could have swept volatile fragments from this trap on to the traps designed to absorb only carbon dioxide. Detection of activity in the carbonate precipitate from trap III, Figure 2, indicates that more traps designed to absorb carbon dioxide would have been desirable. More interesting, however, is the detection of radioactivity in the combined supernatant liquid from the carbonate precipitation. This radioactivity indicates that some of the aflatoxin B_1 was broken down to chloroform-extractable fragments, possibly carbon monoxide. In 1974, Lee et al. reported on mass spectral analysis of the crude ammoniation product of aflatoxin B_1. Nearly 40% of their crude product contained low-molecular-weight compounds or fragments of B_1 (m/e 57–157). It is entirely possible that during ammoniation of meal + B_1,

similar low-molecular-weight volatiles were produced but were not trapped on the system we used.

CONCLUSIONS

In previous reports of chemical analyses, 70% of ammoniated, nonradioactive aflatoxin B_1 was unaccounted for in a model system (Lee et al., 1974; Cucullu et al., 1976). When nonradioactive aflatoxin B_1 was added to peanut meal before ammoniation, the detection of conversion product was even lower, with 99% of the original aflatoxin B_1 unaccounted for after ammoniation (Lee et al., 1978). In the current report, in which radioactive aflatoxin was used, we accounted for 45–50% of the added toxin. Thus, use of labeled aflatoxin B_1 significantly improves the monitoring of its degradation by ammoniation over chemical assays alone. Our results offer sound evidence that the nonvolatile products of ammoniation of B_1 (which include B_1 unchanged by ammoniation) are associated with nonprotein particles. Degradation products are either loosely bound to meal particles and readily soluble in methanol or more tightly bound and not solubilized by methanol. Loss of activity due to volatilization of aflatoxin was not conclusively proven as only a small part of the radioactivity was detected in the volatiles trapped after ammoniation. This report, however, offers the first experimental evidence that some aflatoxin B_1 is reduced to volatiles during ammoniation. Since aflatoxin B_1 is a potent toxicant and carcinogen, further experiments are warranted to effectively trap and determine the nature of the volatiles produced by ammoniation of B_1. More sophisticated studies will have to be designed to determine the nature of binding of aflatoxin or aflatoxin fragments to meal constituents.

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LITERATURE CITED

Beckwith, A. E., Vesonder, R. E., Ciegler, A., *J. Agric. Food Chem.* **23**, 582 (1975).
Biollaz, M., Buchi, G. Milne, G., *J. Am. Chem. Soc.* **92**, 1035 (1970).
Conkerton, E. J., Ory, R. L., Dechary, J. M., *J. Am. Peanut Res. Educ. Assoc.* **5**, 143 (1973).
Cucullu, A. F., Lee, L. S., Pons, W. A., Jr., Stanley, J. B., *J. Agric. Food Chem.* **24**, 408 (1976).
Dollear, F. G., in "Aflatoxins: Scientific Background, Control and Implication", Goldblatt, L. A. Ed., Academic Press, New York, 1969, pp 378–383.
Gardner, H. K., Jr., Koltun, S. P., Dollear, F. G., Rayner, E. T., *J. Am. Oil Chem. Soc.* **48**, 70 (1971).
Lee, L. S., Bennett, J. W., Cucullu, A. F., Ory, R. L., *J. Agric. Food Chem.* **24**, 1167 (1976).
Lee, L. S., Cucullu, A. F., *J. Agric. Food Chem.* **26**, 66 (1978).
Lee, L. S., Stanley, J. B., Cucullu, A. F., Pons, W. A., Jr., Goldblatt, L. A., *J. Assoc. Off. Anal. Chem.* **57**, 626 (1974).
Masri, M. S., Vix, H. L. E., Goldblatt, L. A., U.S. Patent 3429709.
Moore, S., Stein, W. H., *Methods Enzymol.* **6**, 819 (1963).
Natarajan, K. R., Rhee, K. C., Cater, C. M., Matil, K. F., *J. Am. Oil Chem. Soc.* **52**, 44 (1975).
Pons, W. A., Jr., Cucullu, A. F., Franz, A. O., Jr., Goldblatt, L. A., *J. Am. Oil Chem. Soc.* **45**, 694 (1968).

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Chemical Studies on Tobacco Smoke. 63. On the Fate of Nicotine during Pyrolysis and in a Burning Cigarette

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Products obtained from the thermal degradation of [^{14}C]nicotine in a combustion tube (under pyrolytic conditions) and in a cigarette (undergoing machine smoking) were examined by gas–liquid chromatography (GLC), by GLC–mass spectrometry, and by radiochromatography. Under pyrolytic conditions in a combustion tube, nicotine underwent extensive degradation to pyridines, quinolines, arylnitriles, and aromatic hydrocarbons. In contrast, in a burning cigarette, a substantial portion of nicotine remained intact ($\simeq$41%), 12.5% underwent oxidation to CO_2, up to 11% was degraded to volatile pyridine bases, and negligible amounts were converted to neutral or acidic particulate components. A major portion of nicotine and its degradation products was also diverted to sidestream smoke. These results suggest to us that pyrolysis experiments may be of limited value for establishing the fate of nicotine and possibly other tobacco components in a burning cigarette.

Nicotine and compounds derived from it contribute significantly to the organoleptic nature and biological activity of cigarette smoke (Schmeltz, 1971). Therefore, it is important to determine the fate of this major tobacco alkaloid during smoking. Previous studies with endogenous or exogenous nicotine in cigarettes have demonstrated that a substantial portion transfers intact into the smoke stream (Houseman, 1973; Jenkins et al., 1976; Schmeltz et al., 1978). The remaining portion of nicotine undergoes thermal degradation, the extent of which has been alluded to in pyrolysis studies with nicotine (Woodward et al., 1944; Jarboe and Rosene, 1961; Schmeltz et al., 1972; Schmeltz and Hoffmann, 1977) and in studies of smoke from cigarettes containing added nicotine (Bush et al., 1972) or nornicotine (Glock and Wright, 1962). Nevertheless, such studies did not fully describe the pathway of nicotine in a burning cigarette, where several complex processes occur

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