Withdrawal Time Required for Clearance of Aflatoxins from Pig Tissues

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The time necessary to obtain clearance of aflatoxins from the tissues of the pig after removal from a contaminated diet was determined in two trials involving 20 pigs in each. There was a significant reduction in aflatoxin levels in all organs and tissues 1 day after placing the pigs on an aflatoxin-free ration. After 2 days, only one pig contained trace amounts (<0.50 μg/kg) of aflatoxins in the tissues. Four days after removal from the contaminated diet, there were no detectable levels of aflatoxins in any of the tissues. It was also found that a naturally contaminated diet containing only 20 and 31 μg/kg aflatoxins B_1 and B_2, respectively, resulted in traces of aflatoxins B_1, B_2, M_1, and M_2 in the livers and kidneys after 13–14 h withdrawal from the contaminated diet, although none were detected in any other tissues.

Transmission of aflatoxins through animal products (meat, milk, and eggs) to man may result in exposure to these potent carcinogenic compounds (Armbrecht, 1971; Rodricks and Stoloff, 1977). Several research groups (Krogh et al., 1973; Murthy et al., 1975; Hayes et al., 1978; Furtado et al., 1979) have shown that aflatoxins can be deposited in the tissues of the pig, which is confirmed in a review by Stoloff (1979). Thus, meat can provide a dietary source of exposure for humans. Little is known about the length of time required for the pig to metabolize aflatoxins and, thus, clear their tissues of these contaminants.

The present study was designed to determine the length of time necessary to achieve tissue clearance of aflatoxins from pigs fed a contaminated diet. This was accomplished by feeding pigs on an aflatoxin-spiked diet for 42 days and measuring the amount of these toxins and their metabolites in the tissues immediately after removal and comparing them to a similar group of animals fed an unspiked basal diet. The remaining pigs were fed a control diet and pigs from all groups were slaughtered at 1, 2, 4, and 8 days after being placed on this diet to ascertain the length of time necessary to obtain tissue clearance.

MATERIALS AND METHODS

Feeding Trials. Two feeding trials with 20 weanling crossbred pigs in each were carried out by using the same basal ration described earlier by Furtado et al. (1979). Feed and water were allowed ad libitum. The pigs were weighed weekly and feed consumption records were kept. Allotment into groups was based on sex, litter, and weight. In each trial 4 pigs were fed the control (unspiked) diet, and 16 were used to determine the time necessary for tissue clearance. In both trials, the spiked diets contained 551 and 355 μg of aflatoxins B_1 and B_2 per kg of feed, respectively, and the spiked and control (unspiked) rations were fed for 42 days. Then the four control pigs and four of the pigs on the experimental diet were slaughtered within 13–14 h after being taken off feed. The remaining pigs in each trial were placed on an uncontaminated control ration and fed until removed for slaughter at the selected time intervals.

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In trial 1, after the initial feeding period of the spiked diet, the remaining 12 pigs were placed on a control diet and held for 1, 2, 4, 8, 16, and 32 days. At each of these intervals, two pigs were slaughtered for analysis of tissues for aflatoxins. Although we had not previously encountered naturally occurring aflatoxins in the feed, in trial 1 the control diet was found to contain 20 and 31 μg each of aflatoxins B_1 and B_2 per kg of diet, respectively. In trial 2, the same mixed control (unspiked) diet was used as before. As in trial 1, four control pigs and four pigs from the group fed the spiked ration were killed at the end of the 42-day feeding trial, while the remaining 12 animals were fed the uncontaminated diet for either 1, 2, or 4 days before slaughtering 4 of them at each time interval to determine the amount of time necessary for tissue clearance.

Tissue Samples. Immediately after removal from the feeding trial, the pigs were held without feed overnight (13–14 h) and were slaughtered the following morning. The carcasses and organs were examined for gross lesions by a Michigan State Department of Agriculture meat inspector. The kidneys, hearts, spleens, livers, and a sample from the longissimus dorsi muscle were collected, weighed, packaged, frozen, and stored at −20 °C for subsequent aflatoxin analysis.

Extraction and Quantification of Aflatoxins. A modification of the method of Trucksess and Stoloff (1979) was used to extract the aflatoxins from the tissues. After extraction from the tissues with acetone plus aqueous NaCl–citric acid solution and precipitation of the interfering substances with lead acetate solution and ammonium sulfate, the aflatoxin extract was transferred to a silica gel column by using chloroform–hexane (1:1 v/v). Interfering compounds were washed from the column with 100 mL of ether–hexane (3:1 v/v). The aflatoxins were then eluted from the column with 150 mL of chloroform–methanol (97:3 v/v).

Final cleanup and quantification of the aflatoxins present in the meat extract were achieved by using 20 × 20 cm precoated silica gel TLC plates (Sil-G-HR-25, Brinkman Instruments, Inc.), which were cut into either four (10 × 10 cm) or two equal (10 × 20 cm) parts, respectively.

The 10 × 10 cm TLC plates offered a very sensitive and fast test for qualitative identification of aflatoxins in meat tissues. The sample extract was applied in the left lower corner of the plate about 1.5 cm from both edges. The standards were applied in the same direction in the upper left and lower right corners of the plate in 1.0-cm wide strips. The plates were developed in the first direction

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Table I. Summary of Feeding Trials: Means and Standard Deviations

item	trial 1				trial 2			
	control		treated		control		treated	
	mean	SD[a,b]	mean	SD[a,b]	mean	SD[a,b]	mean	SD[a,b]
initial weight, kg	9.5[d]	1.11	9.6[d]	1.14	9.6[e]	0.39	9.6[e]	0.73
final weight, kg	24.7[f]	6.24	20.3[f]	4.04	34.8[g]	1.22	23.1[h]	4.49
weight gain, kg	15.2[i]	6.15	10.7[i]	3.2	25.2[j]	1.11	13.8[k]	4.09
feed intake/pig, kg	38.5		28.5		50.6		34.2	
feed efficiency, kg of feed/kg of gain	2.5		2.7		2.0		2.7	
daily dosage ratio (DR),[c] μg/kg	3		41		0		46	
total aflatoxin intake/pig, mg	2.0		25.8		0		31.0	

[a] SD = standard deviation. [b] Mean values for an item in the same trial followed by different superscripts are significantly different at $P < 0.05$. Where standard deviations are missing, the number of values were not sufficient to allow for statistical analysis. [c] DR = Wa/[0.5(Ws + We)t], where Wa = micrograms of aflatoxins ingested during t (time in days), Ws = starting weight, and We = ending weight in kilograms. Calculations for daily dosage ratio were based on tissue composition obtained as outlined in the USDA (1981) procedures.

with chloroform–acetone (3:2 v/v) in a 1.5-L beaker tightly covered with aluminum foil. After development, the plates were dried as described by Trucksess and Stoloff (1979) and then developed perpendicular to the first direction by using anhydrous diethyl ether–methanol–water (90:8:2 v/v).

The 10 × 20 cm TLC plates were used for quantitative analysis of aflatoxins in the tissues. This technique showed the same accuracy as the 20 × 20 cm plates used by Trucksess and Stoloff (1979), in addition to being less time consuming for development. The plates were scored as shown by Trucksess and Stoloff (1979). The sample spots were applied as described for the 10 × 10 cm plates, and the standards used for the densitometric reading were spotted within three parallel 1.0-cm wide strips toward the longest dimension of the plate. The spots migrated about 5 and 9 cm in the first and second dimension, respectively. The solvent system used for development in the first dimension was either chloroform–acetone (3:2 v/v) or chloroform–acetone-2-propanol (85:10:5 v/v). For development of the plates in the second dimension, a solution of anhydrous diethyl ether–methanol–water (95:4:1 v/v) was used.

The samples were quantitated by using aflatoxin standards for B_1, B_2, M_1, and M_2 prepared by the AOAC (1975) method. Confirmatory tests for aflatoxins were carried out according to the methods of Przybylski (1975) and Trucksess and Stoloff (1979).

Analysis of the feed for aflatoxins was carried out according to the method of AOAC (1975) using a 50-g sample. The extract was spotted on 20 × 20 cm TLC plates. The spots on all plates were quantitated by densitometric analysis using a double-beam scanning–recording–integrating Schoeffel SD 3000-3 spectrodensitometer, with calculations on three replications being made as explained by Furtado et al. (1979).

RESULTS

Feeding Trials. The results of the feeding trials are summarized in Table I. In trial 1, there was no significant difference between the weight gains of the controls and those of the pigs fed aflatoxins. This is in contrast to the earlier study of Furtado et al. (1979) in which pigs fed an aflatoxin-spiked diet gained significantly less than those on an uncontaminated control diet. It also is different than the results of trial 2 in which the weight gains for the control pigs were significantly ($P < 0.05$) higher (45%) than for those fed the aflatoxin-spiked diet (Table I). Furthermore, the control pigs in trial 2 gained 40% more than the controls in trial 1 on the same ration except for the fact that the ration in trial 1 was naturally contaminated with 20 and 31 μg/kg aflatoxins B_1 and B_2, respectively. Although the individual feed components were not analyzed separately, it is probable that the corn used in the corn–soybean meal diet of trial 1 (Furtado et al., 1979) was the source of contamination.

Although the contamination with aflatoxins may have been responsible for the lower weight gains in trial 1, it seems more likely that some other factors may have been involved. The control pigs on the naturally contaminated ration (trial 1, Table I) consumed 23% less feed than similar control pigs on the control diet in trial 2. These results further support the concept that some factor(s) other than the low level of aflatoxins in the control ration from trial 1 may have been responsible for the poor feed intake and growth of the pigs. It is known that a "refusal factor" associated with *Fusarium* infection of corn can result in poor performance upon feeding such corn to swine (Kornegay et al., 1964; Curtin and Tuite, 1966; Vesonder et al., 1973; Thomas et al., 1973).

In trial 1, there was little difference in the feed efficiency between the control and treated pigs, with the corresponding values being 2.5 and 2.7 kg of feed/kg of weight gain. In trial 2, the control pigs fed the uncontaminated diet made more efficient gains than those fed the spiked ration (2.0 vs. 2.7 kg of feed/kg of gain). However, the pigs were group fed so it was impossible to analyze the data for statistical significance. However, the greater efficiency of the control pigs in both trials suggests that the aflatoxins lowered feed efficiency, with the difference being considerably greater in trial 2.

The daily dosage ratio (average aflatoxin intake in micrograms per kilogram live weight) was 3 for the controls in trial 1 in comparison to 41 for the pigs fed the spiked diet. In trial 2, the daily dosage ratio was 0 for the controls on the uncontaminated diet and 46 for the treated group. Thus, there was considerable difference in the daily dosage ratio for the controls and for the treated animals in both trials. Table I also presents the total aflatoxin intake per pig by groups and shows aflatoxin consumption was the greatest for the treated pigs in trial 2, reflecting the greater amount of feed consumption.

Weights of Body Organs. There was little evidence of any gross pathological lesions upon post-mortem examination, except for the livers of two pigs, one from trial 1 at 0 days withdrawal and the other from trial 2 killed after 2 days withdrawal. The livers from these two pigs exhibited a yellowish discoloration, appeared quite fatty and were condemned for human consumption by the meat inspector. Krogh et al. (1973) have reported similar liver lesions in pigs fed aflatoxins over an extended period of time. All other organs were free of visually apparent lesions and were not rejected for human consumption by the meat inspector, even though the livers of treated pigs in

Table II. Summary of Organ Weights Expressed as Percentage of Body Weight

	trial 1				trial 2			
	control		treated		control		treated	
item	mean	SD[a,b]	mean	SD[a,b]	mean	SD[a,b]	mean	SD[a,b]
heart	0.35[c]	0.09	0.43[c]	0.06	0.38[g]	0.03	0.38[g]	0.04
kidneys	0.44[d]	0.10	0.52[d]	0.09	0.49[h]	0.06	0.49[h]	0.07
liver	2.36[e]	0.6	2.92[e]	0.45	2.15[i]	0.25	2.80[j]	0.30
spleen	0.18[f]	0.03	0.18[f]	0.01	0.17[l]	0.02	0.18[l]	0.03

[a] SD = standard deviation. [b] Mean values for an item in the same trial having identical superscripts are not significantly different ($P < 0.05$).

trial 2 were significantly heavier (Table II) than those of the controls. Although the livers of the treated pigs in trial 1 were also heavier on average than those of the controls (Table II), the difference was not significant ($P < 0.05$). This confirms earlier work showing liver enlargement in pigs fed aflatoxins (Keyl and Booth, 1971; Furtado et al., 1979) but differs from other reports (Keyl and Booth, 1971; Armbrecht et al., 1971) claiming kidney enlargements of pigs fed aflatoxins. The failure to observe abnormalities in the kidneys of the aflatoxin-treated pigs in the present study may be related to the short duration of the feeding trial (42 days). Results of the present study do, however, confirm the fact that the liver is the tissue most susceptible to aflatoxin damage and appears to be the best tissue for monitoring the pig's response to aflatoxin damage (Jacobson et al., 1978; Furtado et al., 1979).

In contrast to trial 2, pigs fed the aflatoxin-spiked diet in trial 1 did not have a significant increase in liver weights over the controls (Table II). The low level of aflatoxin contamination on the control diet was apparently high enough to cause some liver enlargement (about 10%) and mask the difference between the two groups. Results of the present study suggest that weanling pigs are very susceptible to aflatoxin toxicity, even at low dietary levels.

Aflatoxin Residues in the Tissues. Levels of aflatoxins in the tissues of pigs slaughtered 13–14 h after removal from the contaminated feed (0 days withdrawal) are presented in Table III. The data show that the control pigs eating the naturally contaminated ration (trial 1) had trace amounts of aflatoxins in their livers and kidneys, but none were detected in the other tissues. This is in contrast to earlier work reporting that aflatoxins fed at levels of 100–300 μg/kg of feed could not be detected in the tissues of pigs (Keyl and Booth, 1971; Monegue et al., 1977), apparently because of the lack of sensitivity of the earlier methods. In fact, Keyl and Booth (1971) reported that they could not detect aflatoxins in the tissues of swine and cattle fed 800 and 1000 μg/kg of feed, respectively. On the other hand, Shreeve et al. (1979) did find measurable amounts of aflatoxins B_1 and M_1 in the milk, urine, and kidneys of cows fed only 20 μg/kg aflatoxin B_1 in the feed. In the present study (Table III), the aflatoxin residues were mainly present as M_1 and M_2, with more being found in the kidneys than the livers. No residues were detected in the heart, spleen, blood, or muscle.

Tissues from most of the pigs on the aflatoxin-spiked ration usually contained measurable aflatoxin residues (Table III). In heart, spleen, blood, and muscle, aflatoxins M_1 and M_2 were present at much lower levels than B_1 and B_2. Blood generally had the lowest level of aflatoxins, followed by muscle, spleen, and heart. Liver and kidney consistently contained more aflatoxins than the other tissues, which may be related to their roles in metabolism and elimination of toxic compounds. Although the mechanism by which these organs remove toxicants from the blood has not been established, active transport and tissue binding are belived to be involved (Klaassen, 1980).

Except in liver and kidney, the M_1 and M_2 residues were much lower than B_1 and B_2. This is probably due to the greater polarity and increased water solubility of these hydroxylated metabolites, making them more easily removed from the tissues as either the original compounds or as their conjugates (Bassir and Osiyemi, 1967; Dalezios et al., 1971). These results are in agreement with the report of Jacobson et al. (1978), who also found that aflatoxins B_1 and M_1 were present at higher levels in kidney and liver than in other tissues.

In contrast to the earlier study by Furtado et al. (1979) in which they reported tentative identification of aflatoxin B_{2a}, the spot was positively identified as M_2 in the present investigation. The earlier identification was not positive due to the unavailability of B_{2a} and M_2 standards. Since these standards were available in the current study, the sample (Furtado et al., 1979) was rechromatographed by using three-dimensional chromatography with three different solvent systems and compared with authentic M_2 and B_{2a} standards. The spot previously identified as B_{2a} was shown to be aflatoxin M_2 and also was consistently found in tissue samples in the present experiment.

Table IV presents the ratios of aflatoxin deposition in the kidneys and livers relative to the levels of B_1 and B_2 in the feed. The data clearly show that B_1 and M_1 are metabolized faster than B_2 and M_2. The value of 648 for the ratio between the level of aflatoxin B_1 in the feed and its level in the liver is lower than the average ratio of 800 calculated by Rodricks and Stoloff (1977). The values are, however, surprisingly close when one considers that the value given by Rodricks and Stoloff (1977) is an average ratio estimated from a number of other studies. The higher ratios for B_2 and M_2 in comparison to those of B_1 and M_1 indicate that the pig metabolizes or clears aflatoxins B_1 and M_1 from the kidney and liver faster than they do B_2 and M_2. The data in Table V demonstrate that the pig deposited only 0.03% of the B_1 from the feed in the tissues compared to 0.04% for B_2. These values are considerably higher than retention values reported from our laboratory earlier (Furtado et al., 1979). The higher retention values obtained in this study are possibly the consequence of using younger pigs.

Tissue Clearance. Results of tissue clearance studies after withdrawal of the aflatoxin-spiked rations are presented in Table VI. At 1 day after removal of the pigs from the spiked diet, low levels to trace amounts of aflatoxins B_1 and B_2 were generally found in all tissues. However, detectable amounts were not found in a single tissue from all pigs. M_1 and M_2 were present in lower amounts than B_1 and B_2 in all tissues and were absent in all samples of heart, spleen, and blood. Only one muscle sample contained a trace amount of M_1.

Two days after removal from the contaminated ration, only traces of B_1 and B_2 were found in any of the tissues of only one of six pigs (Table VI). This pig showed severe liver necrosis, which apparently decreased his ability to metabolize these toxic compounds. Similarly, the liver of

Table III. Levels of Aflatoxins in Pig Tissues 13–14 Hours following Removal from Diets (Micrograms per Kilogram)[a,b]

pig no.	liver				kidney				heart				spleen				blood				muscle			
	B_1	B_2	M_1	M_2	B_1	B_2	M_1	M_2	B_1	B_2	M_1	M_2	B_1	B_2	M_1	M_2	B_1	B_2	M_1	M_2	B_1	B_2	M_1	M_2
Control, Trial 1, Naturally Contaminated																								
1	tr	tr	tr	0.09	tr	tr	0.05	0.20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	tr	tr	tr	0.08	0	0	0.05	0.15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	tr	tr	tr	tr	0	0	0	0	tr	tr	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0.10	0.11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
mean	tr	tr	tr	0.04	0	0	0.05	0.12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Aflatoxin Spiked, Trial 1																								
5	0.31	0.89	0.27	0.28	0.40	1.07	0.29	0.38	0.13	0.15	0	0	0.26	0.14	0	0	0.10	0.14	tr	0	0.20	0.24	0	0
6[c]	0.25	0.41	0	0	0.30	0.45	0.13	tr	0.24	0.44	0.09	0	0.32	0.64	0.15	0	0.17	0.13	0.06	0	0.34	0.50	0.12	0
7	1.52	1.02	0.53	1.08	0.59	0.73	0.39	0.62	0.25	0.33	0.14	0	0.32	0.27	tr	0	0.21	0.17	0.08	0.09	0.45	0.38	0.17	0.14
8	0.36	0.33	0.42	0.57	0.28	0.34	1.10	0.59	0.09	0.14	0.08	0	0.69	0.12	0	0	0.12	0.05	0.10	0	0.18	0.14	0.10	tr
mean	0.61	0.66	0.30	0.48	0.39	0.65	0.48	0.40	0.18	0.26	0.08	0	0.40	0.29	0.04	0	0.15	0.12	0.06	0.02	0.29	0.31	0.10	0.03
Aflatoxin Spiked, Trial 2																								
13	0.86	0.73	0.26	0.67	1.02	0.96	1.13	0.94	0.89	0.42	tr	0	0.51	0.44	tr	0	0.10	0.16	tr	tr	0.28	0.15	0.07	0
14	2.07	1.95	0.19	0.33	0.18	0.21	0.53	1.79	0.20	0.14	tr	tr	tr	tr	tr	0	0.26	0.24	tr	0	0.06	0.05	0.05	0.14
15	0.98	1.06	0.27	0.98	1.14	2.05	0.55	0.60	1.32	1.05	0.09	tr	0.94	0.94	tr	tr	0.56	0.53	tr	tr	0.66	0.60	0.07	tr
16	0.41	0.41	0.33	2.17	0.90	1.45	0.50	0.85	0.62	0.60	tr	tr	0.46	0.48	tr	tr	0.27	0.35	tr	tr	0.46	0.35	tr	tr
mean	1.08	1.04	0.26	1.04	0.81	1.17	0.68	1.04	0.76	0.55	0.02	tr	0.48	0.46	tr	tr	0.30	0.32	tr	tr	0.36	0.29	0.05	0.03

[a] Pig no. 7–12 in trial 2 were controls and had 0 level of aflatoxins in all tissues. [b] tr = traces but less than 0.05 μg/kg of tissues. [c] Pig no. 6 suffered from severe liver necrosis.

Table IV. Ratios of Aflatoxin B_1 and B_2 Levels in Feed in Relation to Their Levels and M_1 and M_2 Levels in the Kidneys and Livers

tissue	aflatoxin in tissue	feed/tissue ratio[a]
kidney	B_1	918
	M_1	605
	B_2	390
	M_2	493
liver	B_1	648
	M_1	1967
	B_2	417
	M_2	467

[a] Higher values indicate more efficient tissue clearance.

Table V. Calculated Amount of Aflatoxins Found in Selected Pig Tissues[a]

tissue	av weight fraction, g	total amount of aflatoxin,[b] μg	
		B_1	B_2
blood[c]	1400	0.35	0.32
heart	74	0.04	0.03
kidneys	92	0.11	0.15
liver	522	0.59	0.84
muscle[d]	8800	3.52	2.99
spleen	33	0.02	0.01
mean % of dosage retained by tissues[e]		0.03	0.04

[a] Data calculated from values in Tables I–III except as noted later. [b] Aflatoxin B_1 is expressed as B_1 equivalents = $B_1 + M_1$. Aflatoxin B_2 is expressed as B_2 equivalents = $B_2 + M_2$. [c] Blood weight is based on average of 22 kg live weight of pig with 6.5% blood (Kornegay et al., 1964). [d] Lean tissue values are based on 22 kg live weight with muscle comprising 40% of live weight (USDA, 1981). [e] Mean tissue retention calculated as percent of dose deposited in the tissues.

one pig slaughtered immediately after removal from the spiked diet (Table III, pig 6) showed severe liver necrosis. The liver of this pig was yellowish in color and appeared to be infiltrated with fat. The absence of M_1 and M_2 in the liver suggests that the pig was unable to metabolize aflatoxins since all other pigs in this group had aflatoxins M_1 and M_2 in their livers.

At 4 days after removal from the aflatoxin-contaminated ration, residues could not be detected in any tissues of any of the pigs (Table VI). The absence of aflatoxins after 4 days on an uncontaminated diet indicates that the pig effectively metabolizes and excretes aflatoxins and readily achieves tissue clearance.

DISCUSSION

The effectiveness of withdrawal of aflatoxin-contaminated feed as a means of attaining tissue clearance from pigs is clearly illustrated by the present study. Rodricks and Stoloff (1977) have pointed out it is not realistic to assume that users of aflatoxin-contaminated products can be subjected to the same strict control required for use of animal drugs. This is especially true in the case of feed ingredients, which may be naturally contaminated without the knowledge of livestock feeders. Nevertheless, the short time required for tissue clearance of aflatoxins in the current investigation is not only an indication that the pig rapidly metabolizes aflatoxins but also is an indicator that after removal from the contaminated feed for a short time (4 days) the pig is relatively safe for human consumption.

The rapid tissue clearance attained in the present study is supported by the recent report of Lüthy et al. (1980), who found that over 50% of radioactive labeled aflatoxin

Table VI. Mean Aflatoxin Residues Detected in Pig Tissues at One, Two, and Four Days following Removal from Contaminated Ration

no. of days after withdrawal	aflatoxin levels, μg/kg of tissue,[a] mean values[b,c]																					
	liver				kidney				heart				spleen				blood				muscle			
	B_1	B_2	M_1	M_2	B_1	B_2	M_1	M_2	B_1	B_2	M_1	M_2	B_1	B_2	M_1	M_2	B_1	B_2	M_1	M_2	B_1	B_2	M_1	M_2
1	0.12 (67)	0.15 (83)	tr (67)	tr (67)	0.13 (83)	0.10 (83)	0.07 (83)	tr (50)	0.89 (67)	0.05 (50)	0 (0)	0 (0)	tr (33)	tr (33)	0 (0)	0 (0)	tr (33)	tr (33)	0 (0)	0 (0)	tr (33)	tr (50)	tr (16)	0 (0)
2	tr 0	tr 0	0 0	0 0	tr 0	tr 0	0 0	0 0	0 tr	tr 0	0 0	0 0	tr tr	tr tr	0 0	0 0	0 0	tr tr	0 0	0 0	0 0	tr 0	0 0	0 0
4[d]	(16) 0 (0)	(16) 0 (0)	(0) 0 (0)	(0) 0 (0)	(16) 0 (0)	(16) 0 (0)	(0) 0 (0)	(0) 0 (0)	(0) 0 (0)	(16) 0 (0)	(0) 0 (0)	(0) 0 (0)	(16) 0 (0)	(0) 0 (0)	(0) 0 (0)	(0) 0 (0)	(16) 0 (0)	(16) 0 (0)	(0) 0 (0)	(0) 0 (0)	(0) 0 (0)	(16) 0 (0)	(0) 0 (0)	(0) 0 (0)

[a] Determination limits were approximately <0.05 μg/kg. Traces (tr) could be seen but not quantitated. [b] Mean values were given as tr (traces) if any of the tissues showed traces. [c] Number of tissue examined having the aflatoxin is in parentheses. [d] Values for all tissues were 0 after 4 days or more.

B_1 was excreted in the feces and an additional 20% in the urine within 9 days of administration to the pig. Furthermore, only 4% of the radioactivity in the feces and urine was found to be in readily identifiable forms, mainly as aflatoxins M_1 and B_1 per se and traces of aflatoxicol. Earlier work on in vivo covalent binding of aflatoxin metabolites led Jaggi et al. (1980) to conclude that macromolecular bound aflatoxin B_1 was at least 4000 times less active than B_1 per se with respect to binding to rat liver DNA, whereas the water-soluble conjugates were at least 100 times less potent than aflatoxin B_1 itself. On this basis, they concluded that the risk to humans consuming liver or meat containing aflatoxin residues is negligible compared to that from consuming other aflatoxin-contaminated foods, such as nuts or milk.

Considerable evidence suggests that aflatoxin B_1 2,3-oxide is the ultimate potent heptocarcinogen derived from aflatoxin B_1 (Swenson et al., 1977; Lin et al., 1978). Thus, aflatoxin B_1 per se would provide the most direct pathway for formation of the B_1 epoxide and would be a more likely contributor to carcinogenicity than B_2 or M_1. In the present study, M_1 was shown to be present in the tissues of the pig and would presumably be less potent than B_1 according to estimates of Hayes (1980).

Rodricks and Stoloff (1977) calculated that aflatoxin carry-over in dairy cows from the feed to milk was about 300:1, which can be compared a value of 648:1 for pigs in the present study. In a recent investigation by Patterson et al. (1980), it was found that about 2.2% of the aflatoxin B_1 ingested was carried over into the milk of cows, although there was considerable variation from animal to animal. Thus, the feed to milk ratio for dairy cows may be considerably higher than the earlier estimate made by Rodricks and Stoloff (1977). The higher values are undoubtedly due to better methods for detecting and measuring aflatoxins. The less-sensitive methods used earlier could account for the discrepancies in some reports in which aflatoxins were not found in milk after replacement of an aflatoxin-contaminated diet with uncontaminated feed after 48 (Polan et al., 1974) or 72 h (McKinney et al., 1973), in contrast to those reporting that aflatoxins were still present after 7 days on an aflatoxin-free ration (Keyl and Booth, 1971).

Results of the present study demonstrate that the pig rapidly metabolizes and clears its tissues of aflatoxins on feeding an uncontaminated diet. This adds further support to the conclusions of Jaggi et al. (1980) that the risk from eating liver or meat of animals fed an aflatoxin-contaminated diet are negligible.

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Toxic 12,13-Epoxytrichothecenes from Anise Fruits Infected with *Trichothecium roseum*

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Four 12,13-epoxytrichothecenes, viz., 4-*O*-acetyltrichothecolone (**1**), 4-*O*-cinnamoyltrichothecolone (**2**), trichothecolone (**3**), and trichothecin (**4**), were isolated and characterized from anise fruits infected in fields with *Trichothecium roseum* van Beyma (CMI-IMI 225229). Among these compounds, **1** and **2** are naturally occurring new 12,13-epoxytrichothecenes. From the in vitro culture extracts of the fungus, only **1**, **3**, and **4** were isolated and identified. Thus, the host–parasite interaction was suggested for the formation of **2**, its cinnamoyl moiety being a metabolite of the host species. The total chloroform extractives of the moldy anise fruits produced dermatitic skin reactions in albino rats on external application and caused listlessness, anorexia, diarrhea, and stunted growth when ingested. The circumstantial evidence suggests toxin risk in man from prolonged ingestion of moldy anise fruits.

In connection with our work on mycotoxins in food and feed materials (Ghosal et al., 1976, 1979a,b), the fungal infection of anise (*Pimpinella anisum* Linn.; family, Umbelliferae) in fields was detected for the first time. Anise is widely cultivated in India in the northern parts, in Uttar Pradesh, and in Orissa, and is liberally consumed by Indians as a chewing spice, in food preparations, and also in medications. Extract of anise fruits is used in Ayurvedic system of medicine (Chopra et al., 1956) as a diuretic, a carminative in the treatment of colic, and, in combination with licorice roots, an anti-ulcerogenic agent (Revers, 1946). During a field survey, by the present authors in 1977, in the Mirzapur District of Uttar Pradesh, fruit-bearing anise herbs were found to be heavily infested with a fungus. The fungus was isolated from the moldy anise fruits by the single-spore isolation method (Funder, 1961) and was identified as *Trichothecium roseum* van Beyma. The identity of the strain (CMI-IMI 225229) was confirmed by the Commonwealth Mycological Institute, Kew, England.

Although report of any toxicosis in humans or animals ingesting moldy anise is not known so far, this investigation was thought warranted for a number of reasons: (1) highly toxic isolates were reported from *T. roseum* (Joffe, 1971); (2) *T. roseum* species is a well-known producer of 12,13-epoxytrichothecenes (Achilladelis and Hanson, 1969); (3) 12,13-epoxytrichothecenes, on prolonged ingestion, are known to produce degeneration of nerve cells in the brain and central nervous system of laboratory animals (Tatsuno, 1968); (4) initial experiments with a chloroform extract of the moldy anise fruits, in the authors' laboratory, suggested the presence of 12,13-epoxytrichothecenes when examined according to a published procedure (Ghosal et al., 1978). Albino rats when fed in their diet with the chloroform extractives of the moldy anise fruits developed anorexia and diarrhea. Common people in all parts of India consume sizable amounts of anise fruits (ranging from ca. 0.1–1 g person^{-1} day^{-1}) in different preparations. Prolonged ingestion of moldy anise may produce neurological disorders or predispose man to other ailments. Chemical and

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