Transmission and Distribution of Aflatoxin in Contaminated Beef Liver and Other Tissues

R.D. STUBBLEFIELD and **O.L. SHOTWELL**, Northern Regional Research Center, Agricultural Research, Science and Education Administration, USDA, Peoria, IL 61604, and **J.L. RICHARD** and **A.C. PIER**, National Animal Disease Center, Science and Education Administration, USDA, Ames, IA 50010

ABSTRACT

A 160-kg Holstein steer was fed 52 mg B_1 equivalents/day (orally) for 5 consecutive days and then slaughtered. The liver, kidneys, spleen, heart and skeletal muscle (round) were retained and assayed for aflatoxin. The liver was cut into 14 samples and each was analyzed to determine the distribution of aflatoxin in liver. Aflatoxin levels in samples from the edges of the liver were 44% lower than averages of all the remaining samples, which were 25.0 ng B_1, 15.4 ng M_1 and 47.1 ng total aflatoxin/g; however, cross-sectional samples would give representative assay results. Lower total aflatoxin concentrations (16.0 ng, 18.5 ng and 12.9 ng/g) were found for heart, spleen and muscle, respectively. The kidneys had the highest level of aflatoxin (145.9 ng/g) with M_1 levels (105.5 ng/g) 3.6 times the B_1 concentration (29.3 ng/g). In the other tissues, B_1 concentrations were 1.6-2.9 times greater than aflatoxin M_1.

INTRODUCTION

Several reviews (1-3) have summarized aflatoxin B_1 and M_1 concentrations detected in edible meat tissues, milk and eggs. In general, a major portion of the ingested aflatoxin is excreted in animal wastes within 24 hr, and small quantities are retained in tissues. The liver is the target organ of aflatoxicosis and has been the subject of most investigations; consequently, very little data is available on concentrations of aflatoxin in other edible tissues, especially from cattle.

Large-animal feeding studies are expensive because of animal costs and the cost of preparing or purchasing large quantities of purified aflatoxin. Keyl and Booth (4) reported in 1971 that there were no deleterious effects on cattle consuming 300 ppb aflatoxin in feed. Cattle that ingested feeds containing 700 ppb or more experienced decreased feed efficiencies and weight gains, but no aflatoxins were found in the edible meats even at these levels. Most of the cattle-feeding studies were done prior to 1976; since then, more accurate and sensitive methods (5-8) ($\leq$0.1 ng/g) for animal tissues have been developed. It is common knowledge that field crops (corn, cottonseed and peanuts) in some areas can become contaminated with aflatoxin (9). Although these crops are being diverted from the consumer markets, they are being fed to farm animals, as evidenced by aflatoxin M_1 occurrence in milk (2). Therefore, it is important to examine the edible tissues of cattle with current sensitive methods to see if toxic residues remaining in tissues are a potential hazard.

One of the objectives of this study was to determine if aflatoxin is uniformly distributed in a steer liver or if special steps are necessary to obtain a representative assay sample. During our study, Stoloff and Trucksess (10) reported that aflatoxin was evenly distributed in pig and calf livers based on several cross-sectional slices. Our other objective was to determine the presence or absence of aflatoxins in bovine heart, kidneys, spleen and skeletal muscle (round). A joint research effort between the National Animal Disease Center (NADC) (Ames, IA) and the Northern Regional Research Center (NRRC) (Peoria, IL) was initiated to accomplish these research objectives. The steer dosing and slaughtering were done at NADC, and tissue analysis was done at NRRC.

EXPERIMENTAL

A 6-month Holstein-Freisian steer (ca. 160 kg body wt) that had not been challenged with aflatoxin was fed a balanced calf ration (NADC 550) before dosing. Partially purified aflatoxins B_1, B_2, G_1 and G_2 (53.7% B_1, 23.3% G_1 and undetermined B_2 and G_2) were weighed into 5 gelatin capsules (80 mg/capsule). Each capsule contained 52 mg B_1 equivalents as computed and described by Richard et al. (11). One capsule was administered orally for 5 consecutive days to give a subacute toxic dose of 0.33 mg aflatoxin/kg body wt/day.

On the 6th day, the steer was slaughtered, and the liver, kidneys, heart, spleen and skeletal muscle (round) were retained. The tissues were cooled (4 C) overnight, iced, taken to NRRC, weighed and all organs except liver were stored at -20 C until assayed. The liver was cut into sections as shown in Figure 1, before freezing, to determine the aflatoxin distribution. Each organ was thawed overnight at 4 C, and sections were either blended or ground in a food grinder for assay. Aliquots (100-g, when possible) were extracted and analyzed by the method of Stubblefield and Shotwell (8). Confirmation of identity of aflatoxins B_1, G_1 and M_1 was done by the method of van Egmond and Stubblefield (12).

RESULTS AND DISCUSSION

Aflatoxin concentrations in the 14 samples taken from the liver are given in Table I. The mean B_1 concentration was 25.0 ng/g, and the mean M_1 concentration was 15.4 ng/g.

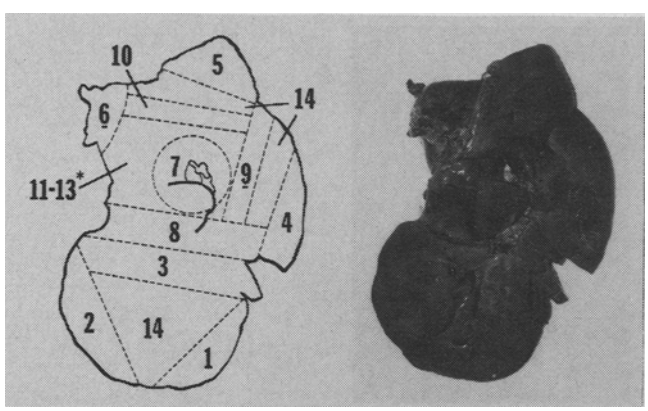

FIG. 1. (*Right*) Picture of liver from 160-kg Holstein steer fed partially purified aflatoxins B_1, B_2, G_1 and G_2 at a level of 52 mg B_1 equivalents/kg body wt/day for 5 days. (*Left*) Diagram depicting individual samples to determine the distribution of aflatoxin. Numbers 11, 12 and 13* are samples of progressive 1.0-1.5-cm layers (*top to bottom*) near the hialus (no. 7).

TABLE I

Aflatoxin Concentrations in Contaminated Beef Liver[a]

Liver sample	Weight (g)	Aflatoxin concentration[b] (ng/g)					
		B_1	B_2	G_1	G_2	M_1	Total
1[c]	40.9	8.1	1.6	0.4	<0.03	3.2	13.3
2[c]	66.2	17.1	2.6	1.3	<0.03	10.6	31.6
3	132.1	23.1	3.8	2.4	0.3	14.4	44.0
4[c]	51.3	19.6	3.3	1.6	0.1	11.6	36.3
5	84.3	25.1	4.1	1.6	0.2	19.5	50.5
6[c]	53.3	14.1	2.2	0.7	<0.03	8.5	25.5
7	136.9	28.8	4.5	3.4	0.1	13.8	50.6
8	162.8	21.8	3.9	1.8	0.2	16.5	44.2
9	89.7	24.7	4.4	2.4	0.1	10.3	41.9
10	78.4	23.6	4.0	1.7	<0.03	16.6	45.9
11[d]	101.0	25.3	3.9	2.2	0.1	15.1	46.6
12[d]	97.1	28.6	4.5	2.8	0.1	12.9	48.9
13[d]	133.8	28.7	4.6	2.6	0.2	19.7	55.8
14	977.8	25.4	4.3	2.9	0.2	15.6	48.4
$\bar{X}$		25.0	4.2	2.3	0.2	15.4	47.1
Std. dev.		2.3	0.3	0.6	0.1	2.7	3.4
N		8	8	8	8	8	8

[a]From 160-kg Holstein steer fed 52.5 mg B_1 equivalents/day for 5 days. Samples cut as depicted in Fig. 1.

[b]As determined by the method of Stubblefield and Shotwell (8).

[c]Values not included in statistics.

[d]Layers of tissue 1.0, 1.0, 1.5 cm thick, respectively, near the hialus (no. 7). Mean values of 11, 12, 13 used in statistics.

Smaller quantities of B_2, G_1 and G_2 were present to give a total aflatoxin concentration of 47.1 ng/g in the liver. Four samples from the liver edges had low values (ca. 56% average): 1, 2, 4 and 6 (Fig. 1). Sample 5 is the small lobe of the liver and not an edge. The 3 vertical layer samples (no. 11, 12 and 13) near the hialus (no. 7) had slightly higher concentrations but were within the standard deviation of the method (8). The data indicate that any cross-sectional sample taken from the liver would give a representative sample, but an edge sample would give low results. This conclusion agrees with that of Stoloff and Trucksess (10); however, these workers did not examine edges of the liver.

To determine if sample size was an important factor and contributed to the low results from the edge samples, smaller quantities (10 g) of sample 14 were reassayed (12 months later). Values of 26.1 ng B_1 and 8.8 ng M_1/g were found (not shown). The M_1 level had decreased ca. 45%. Sufficient sample remained to reassay a 100-g portion, and the M_1 concentration (9.9 ng/g) showed a comparable decrease over the 12-month period. The data suggest that quantity is not a critical factor for representative sampling, but the larger 100-g portions are preferred to ensure detection of low toxin levels. The data also indicated that degradation or binding of M_1 occurred over a period of time even when the tissue was frozen. Therefore, meat analysis should be done as quickly as possible for maximal detection of aflatoxin.

Table II gives the aflatoxin concentrations in the other organs. All 5 aflatoxins were found in the tissues, except that G_2 was not detected in the spleen. The lower total aflatoxin content in the spleen, heart and skeletal muscle (18.4, 16.0 and 12.9 ng/g, respectively) when compared to liver was expected (1-3); however, the very high concentration of aflatoxin (145.9 ng/g) in the kidney was surprising. The kidney B_1 (29.3 ng/g) is similar to that in liver (25.0 ng/g, Table I), but the kidney M_1 level (105.5 ng/g) is nearly 7 times the M_1 in liver (15.4 ng/g). For all other tissues, B_1 concentrations were 1.6-2.9 times greater than aflatoxin M_1; but in kidneys, the M_1 was 3.6 times greater than B_1. A similar ratio (3:1) in kidney has been reported (1), but the concentrations were much lower than in this study (0.23 ng B_1 and 0.72 ng M_1/g).

The reason for the high aflatoxin content in kidney is unclear. Residual urine would account for a slightly increased M_1 value, but it would not contribute significantly. Also, aflatoxin M_1 would accumulate in the kidneys if it is not readily dissipated into the urine. Another explanation could be that the kidney enzyme system is metabolizing B_1 from the blood stream to form higher levels of M_1. The liver metabolism of B_1 to form M_1 and other metabolites has been studied extensively in rats and other small animals;

TABLE II

Aflatoxin Concentrations in Contaminated Bovine Tissues[a]

Tissue	Weight (g)	Aflatoxin concentration (ng/g)[b]					
		B_1	B_2	G_1	G_2	M_1	Total
Heart	852.3	9.2	0.9	1.0	0.04	4.9	16.0
Spleen	381.5	12.2	1.6	0.4	0	4.3	18.5
Muscle (round)	1064.5	7.9	1.1	1.1	0.05	2.7	12.9
Kidney	627.2	29.3	4.4	6.3	0.4	105.5	145.9

[a]From Holstein steer (160-kg) fed 52 mg B_1 equivalents/day for 5 consecutive days.

[b]As determined by the method of Stubblefield and Shotwell (8). Each value is an average of 3 or more determinations.

however, the authors have not found any published reports of kidney metabolism studies. This is an area that should be investigated.

The amount of aflatoxin B_1 fed to the steer in this study is considerably higher than the amount fed in other cattle studies (1-3). However, if a 160-kg calf, which would normally consume 2½-3% of its weight in feed/day (4-4.8 kg), ate contaminated feed at 500 ng/g, detectable levels of B_1 and M_1 should be found in tissues with currently available methods. This presumes that toxin transmission to tissue is proportional to the toxin ingested after a certain level of aflatoxin has been reached. According to the data of Keyl and Booth (4), aflatoxin is detrimental to cattle at a concentration between 300 and 700 ppb. Consequently, measurable quantities of aflatoxins are probably transmitted to the meat at those levels, also. It is obvious that there is still much information to be learned about the transmission of aflatoxin to edible tissues of large animals such as cattle.

ACKNOWLEDGMENTS

The authors thank J.A. Greer (NRRC) for analysis of tissues, R. Fichtner (NADC) for technical assistance and H.P. van Egmond (National Institute of Public Health, Bilthoven, The Netherlands) for his valuable cooperation.

REFERENCES

1. Rodricks, J.V., and L. Stoloff, in "Mycotoxins in Human and Animal Health," edited by J.V. Rodricks, C.W. Hesseltine and M.A. Mehlman, Pathotox Publishers, Inc., Park Forest South, IL, 1977, pp. 67-79.
2. Stoloff, L., in "Interactions of Mycotoxins in Animal Production," proceedings of a symposium jointly sponsored by American Society of Animal Science, American Dairy Science Association, and Committee on Animal Nutrition/National Research Council, at Michigan State University, July 1978, pp. 157-166.
3. Edds, George T., in "Conference on Mycotoxins in Animal Feeds and Grains Related to Animal Health," sponsored by Bureau of Veterinary Medicine/Food and Drug Administration at FDA, Rockville, MD, June 1979, pp. 80-164.
4. Keyl, A.C., and A.N. Booth, JAOCS 48:599 (1971).
5. Jemmali, M., and T.R.K. Murthy, Z. Lebensm. Unter-Forsch. 161:13 (1976).
6. Trucksess, M.W., and L. Stoloff, J. Assoc. Off. Anal. Chem. 62:1080 (1979).
7. van Egmond, H.P., W.E. Paulsch, E.A. Sizoo and P.L. Schuller, Fourth International IUPAC Symposium on Mycotoxins & Phycotoxins, Lausanne, Switzerland, 1979, abstr.
8. Stubblefield, R.D., and O.L. Shotwell, J. Assoc. Off. Anal. Chem. (in press).
9. Tuite, J., in "Interactions of Mycotoxins in Animal Production," proceedings of a symposium jointly sponsored by American Society of Animal Science, American Dairy Science Association, and Committee on Animal Nutrition/National Research Council, at Michigan State University, July 1978, pp. 19-39.
10. Stoloff, L., and M.W. Trucksess, J. Assoc. Off. Anal. Chem. 62:1361 (1979).
11. Richard, J.L., J.R. Thurston, E.B. Lillehoj, S.J. Cysewski and G.O. Booth, J. Am. Vet. Res. 39:163 (1978).
12. van Egmond, H.P., and R.D. Stubblefield, J. Assoc. Off. Anal. Chem. 64:152 (1981).

Hepatotoxicity of the Mycotoxin Penicillic Acid: A Pharmacokinetics Consideration

P.K. CHAN and **A.W. HAYES**, Toxicology Department, Rohm and Haas Company, 727 Norristown Road, Spring House, PA 19477

ABSTRACT

The hepatotoxicity of penicillic acid (PA), a carcinogenic mycotoxin, was substantiated by a variety of hepatic functional tests. Involvement of an active metabolite as the toxic species was proposed. The toxicity of PA was dependent on the route of administration with intraperitoneal (ip) being the most toxic followed by intravenous (iv) and oral. This difference in toxicity was explained by the kinetic data for PA if liver were assumed to be the site of activation. One-, 2- and 3-compartment open models were proposed to fit the plasma parent compound concentration after oral, ip, and iv administration of PA. Liver, kidneys, heart, lungs and spleen contained more radioactivity than brain, fat and muscle after [^{14}C]-PA administration. Only a fraction of the radioactivity in the blood was detected as the parent compound. Most of the recovered radioactivity in the kidneys and liver was in the cytosol fraction. [^{14}C]PA was readily metabolized in the liver. The metabolites were excreted in the bile and effectively cleared by the kidneys. Fecal and respiratory CO_2 were minor excretory routes. Over 90% of the urinary and 99% of the biliary metabolites were not extracted with polar organic solvents. Three water-soluble metabolites (derived from GSH or cysteine) were resolved by HPLC in urine and bile. About 10% of the urinary metabolites were detected as glucuronide conjugates. These data supported the hypothesis that an active metabolite which can be detoxified by GSH is involved in the toxicity of PA.

INTRODUCTION

Penicillic acid (PA), an α,β-unsaturated conjugated lactone, is produced by several food-borne fungi (1). Since the discovery of its carcinogenicity in experimental animals (2-4) and its subsequent isolation from agricultural products (5-9), PA has been considered a potential environmental health hazard to man. In addition to being carcinogenic, PA is hepatotoxic in experimental animals. For example, PA caused generalized hepatocyte necrosis in mice (10) and sinusoidal congestion in caine liver (11). Morphologic damage also occurred in hepatocytes treated with PA concentration as low as 1×10^{-5} M (12). The hepatotoxicity of PA was substantiated by studies which showed that PA increased pentobarbital-induced sleeping time while decreasing in vivo and in vitro pentobarbital metabolism (13); decreased hepatic reduced glutathione (GSH), elevated serum transaminase and bilirubin, and increased sulfobromophthalein and indocyanine green retention (14); and depressed hepatic biliary excretion of indocyanine green (15). The involvement of an active metabolite and the protective role of GSH in PA hepatotoxicity was proposed (13,14). The acute toxicity of PA was increased in enzyme-induced or GSH-depleted mice, but decreased in enzyme-inhibited mice or when the GSH level was maintained by cysteine pretreatment (13,14). Based on these results, a metabolic pathway by which PA could be detoxified by GSH was proposed. Pharmacokinetic data needed to support or negate this hypothesis, however, were not available.