Metabolism of Aflatoxin B₁ in Rhesus Monkeys

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SUMMARY

The metabolism of aflatoxin B₁ has been investigated in male rhesus monkeys given i.p. injections of a mixture of ring-labeled aflatoxin B₁-¹⁴C and nonradioactive toxin, at a total dose of 0.4 mg/kg. Thirty-five % of the administered radioactivity was excreted in urine within 96 hr. The excretion rate reached a maximum 1 hr after dosing and then fell rapidly, but urine excreted after 96 hr still contained detectable radioactivity.

A new method for isolation and identification of urinary aflatoxin metabolites was devised. Of the total urinary metabolites, only 12 to 15% were chloroform extractable, including at least 3 fluorescent metabolites of unknown structure in addition to aflatoxins B₁ and M₁. Chloroform-insoluble metabolites were isolated on an Amberlite XAD-2 column and separated by a DEAE-Sephadex A-25 column into 6 subfractions. The major subfraction (60%) was hydrolyzed almost quantitatively by β-glucuronidase and the liberated metabolite was identified as aflatoxin P₁, the O-demethylation product of aflatoxin B₁. Aflatoxin P₁ in urine represented approximately 20% of the administered dose, 17% as glucuronide, 3% as sulfate, and 1% as unconjugated phenol. Aflatoxins M₁ and B₁ in urine accounted for 2.3 and 0.01 to 0.1% of the dose, respectively.

Four days after injection, 5.6% of the aflatoxin dose was still retained by liver and was mainly bound to liver proteins. Radioactivity in blood attained a peak at 1 hr after injection, and at 96 hr it decreased to 40% of its maximum value. Gel electrophoresis of serum followed by autoradiography indicated association of the radioactivity with the albumin fraction.

INTRODUCTION

Various aspects of the metabolic fate of aflatoxin B have been investigated in animals. Aflatoxin M₁, a hydroxylated derivative, has been identified by fluorescence techniques as a metabolite appearing in the milk and urine of cows, sheep, and rats dosed experimentally with B₁ (see Ref. 21 for review). It has also been found in the urine of humans ingesting aflatoxin-contaminated diets (4). In animals and humans, only about 5% of ingested B₁ appears to be excreted as M₁ in urine (4, 21). The kinetics of aflatoxin distribution and excretion in rats has been quantitatively studied with the use of radioactive aflatoxin B₁ (22). These studies indicated urinary excretion of substantially larger amounts of aflatoxin derivatives than could be accounted for by aflatoxin M₁ and also that O-demethylation constituted a major pathway of metabolic conversion.

Knowledge of the metabolism of the toxin is important to an understanding of the factors determining response and also for extrapolation of animal data to evaluate human risk. We therefore undertook investigations of the pharmacokinetics of aflatoxin B₁-¹⁴C in rhesus monkeys with the following objectives: developing methodology adequate for separation and isolation of urinary metabolites; determining the quantitative relationships between excretion of metabolites and administered dose; and determining the distribution of injected aflatoxin B₁ in tissues and blood.

Monkeys were selected for these investigations in order to obtain information on urinary excretory patterns in a primate that could subsequently be compared with observations in other species, ultimately including man. Development of methodology for quantitative recovery of urinary metabolites was greatly facilitated by the use of these animals. In the course of these experiments, aflatoxin P₁ was isolated and identified as a major urinary metabolite, as we have reported elsewhere (7). This paper summarized findings of other phases of the investigation.

MATERIALS AND METHODS

Male rhesus monkeys were provided by and housed at the New England Regional Primate Research Center, Southboro, Mass. They weighed 2.5 to 4.0 kg and were maintained on a Purina chow diet supplemented with fresh fruit. During metabolism experiments, the animals were kept in a restraining chair. For facilitation of quantitative timed urine collection, water was withheld overnight; then access to it was provided 2 hr before each experiment began. The animals were given food throughout the course of the study period, and urine was quantitatively collected, without fecal contamination, in a vessel submerged in ice and protected from light.

Aflatoxin B₁, labeled with ¹⁴C in the ring carbons (1), was prepared from acetate-1-¹⁴C according to the method of Donkersloot et al. (8), the specific activity of the final preparation being 29.6 μCi/μmole. Purity of the preparation was verified by thin-layer chromatography on Silica Gel GHR (Brinkmann Instruments, Inc., Westbury, N. Y.) plates developed with chloroform:acetone, 9:1 (v/v). Autoradiography and exposure to long-wave UV light showed no contamination by other substances within a detection limit of 1.6 X 10⁻² μCi. Nonradioactive aflatoxin B₁ was isolated by chromatographic fraction of culture extracts. Dimethyl sulfoxide (spectral grade; Burdick and Jackson Laboratories, Muskegon, Mich.) was used as the aflatoxin vehicle. Other
chemicals and sources included: Amberlite XAD-2, a neutral cross-linked polystyrene polymer (Rohm and Haas Co., Philadelphia, Pa.); DEAE-Sephadex A-25 (Pharmacia Fine Chemicals, Inc., Piscataway, N. J.); Adsorbosil 1 (Applied Science Laboratories, Inc., State College, Pa.); MN-Silica Gel GHR and Silica Gel 0.05 to 0.2 mm (Brinkmann Instruments, Inc., Westbury, N. Y.); β-glucuronidase (Ketodase) (Warner-Chilcott Laboratories, Morris Plains, N. J.); β-glucuronidase (type I) and arylsulfatase (Type III) (Sigma Chemical Co., St. Louis, Mo.); Mylase P (analytical grade) which, according to the work of Hobkirk et al. (12), contains phenol sulfatase (Schwarz/Mann, New York, N. Y.); PPO, POPOP, and M2POPOP, scintillation grade (Packard Instrument Co., Downers Grove, Ill.)

**Experimental Protocols.** Four male monkeys were each given a single i.p. injection of a mixture containing about 5 μCi radioactive aflatoxin B₁, together with nonradioactive toxin at a dose level of 0.4 mg/kg body weight, dissolved in 0.8 ml dimethyl sulfoxide. Time of dosing was 9:30 to 10:00 a.m. Immediately after dosing, timed urine collection was initiated and was continued until the animal was killed. Samples were immediately frozen and kept in the dark until processed, but in no case did the storage period exceed 24 hr. Specific radioactivity of each urine sample was determined by liquid scintillation counting in triplicate aliquots (3). All radioactive measurements were made in a liquid scintillation spectrometer with toluene-14C as the internal standard. Samples of 3 to 5 ml blood were taken at time intervals of 10 min and 1, 6, 24, 48, 72, and 96 hr after dosing. Triplicate aliquots from each sample were counted for radioactivity by the method of Hansen and Bush (11). Blood was separated into plasma and packed cells by centrifugation; cells were washed by resuspension in 0.9% NaCl solution, and then lyzed by the addition of distilled water. Proteins of plasma and cells were precipitated with 4 volumes ethanol, extracted (20), and counted.

Monkeys were killed at 45 min and 24, 48, and 96 hr after i.v. barbiturate overdosing. The liver was perfused in situ with cold 0.9% NaCl solution, and visceral organs were immediately excised, washed, and chilled. Organs were frozen, stored for about 24 hr, and then homogenized in alcoholic KOH, solubilized with Soluene 100, and neutralized with 5 N HCl. Radioactivity was determined by liquid scintillation counting. The liver was homogenized and subjected to chemical analysis and isolation of liver glycogen, protein, and lipids (18).

**Urine Extraction Procedure.** All manipulations were done either in darkness or in subdued light in order to minimize photolysis of metabolites. Samples were pooled according to the time of excretion: Fraction 1 (0 to 2 hr); Fraction 2 (3 to 48 hr), and Fraction 3 (49 to 96 hr). Extractions were carried out with equal volumes of urine and solvent, in a mechanical shaker (Extracto-Matic; VirTis Co., Gardiner, N. Y.). Urine was successively extracted 7 times for 15 min each, first with a mixture of methanol:chloroform (6:4, v/v) and then with chloroform alone. Extracts were pooled, reduced in volume in a flash evaporator at 25°, and finally evaporated to dryness under nitrogen. The dry residue was dissolved in a minimum volume of chloroform, and 5 to 20 μl were applied to thin-layer chromatography plates to separate individual metabolites.

**Thin-Layer Chromatography.** Two chromatographic systems (10, 19) were equally satisfactory for separation and detection of the isolated metabolites of aflatoxin B₁. Radioactive metabolites were identified by autoradiography of chromatograms, for which X-ray film, developed after exposure of 1 week, was used.

**Isolation and Separation of Aflatoxin Conjugates.** Solvents retained in the aqueous phase were removed by flash evaporation at 30°. A column (4.1 x 60 cm) was packed with 450 g Amberlite XAD-2 according to the procedure described by Bradlow (2) for isolating steroid conjugates. Fractions containing aflatoxin conjugates were recycled through the resin by gravity flow for 1.5 hr. The column was drained, washed with 400 ml water, and allowed to drain completely. Conjugates (95% or more of radioactivity in aqueous phase) retained on the resin were eluted by 5 successive 300-ml portions of methanol. The 5 methanol fractions were pooled and reduced to dryness at a temperature of less than 35°. The residue of conjugates was then dissolved in distilled water.

A K15/90 Sephadex column was packed with 16 g DEAE-Sephadex A-25 to form an 80-cm column. Samples of 3 to 4 ml of conjugate residues prepared as above were applied to the column, and elution was carried out with a linear 0.0 to 0.8 M NaCl gradient (12). Fractions of 10 ml were collected, and the specific activity of each fraction was determined by scintillation counting.

**Enzymatic Hydrolysis Procedures.** Aliquots of conjugates recovered from the Amberlite column and radioactive fractions resolved by the DEAE-Sephadex A-25 column were desalted by recycling through a small (1.5 x 20 cm) Amberlite column and elution with ethanol. They were incubated with enzyme preparations at 37° for 48 hr in 0.2 M acetate buffer, pH 5.0. These mixtures were then extracted with chloroform:methanol as described for urine extraction. The enzyme preparations used were Ketodase, arylsulfatase, and Mylase P. The arylsulfatase preparation possessed both sulfatase and some glucuronidase activity. For achievement of selective sulfatase activity, conjugates were incubated first with Ketodase and the incubation mixture was extracted as described above. The aqueous residue remaining thereafter was reconstituted to its original volume with acetate buffer, sulfatase was added, and the mixture was again incubated at 37° for 48 hr. The incubation mixture was extracted, concentrated, and subjected to thin-layer chromatography as described above. Purification of aflatoxin P₁ was achieved by a procedure involving silica gel columns previously used for isolating aflatoxins B₁ and M₁ (15).

**RESULTS**

**Urinary Excretion Pattern.** In the 96-hr period after a single i.p. injection of aflatoxin B₁, the monkeys excreted approximately 35% of the dose in urine. Chart 1 shows that excretion of radioactivity was most rapid during the 1st 2 hr, during which 16% of injected radioactivity was found in urine. Urine excreted during the 1st 24-hr interval contained 26 to
30% of the dose, the remainder appearing by 96 hr. Excretion rates for all 3 monkeys were almost identical, reaching a maximum about 1 hr after dosing and then falling rapidly, approaching 10% of the maximum value within 4 hr; urine still contained detectable radioactivity 96 hr after dosing.

**Chloroform-soluble Metabolites.** Only 15% of the total radioactivity in urine was extractable with chloroform, and thin-layer chromatography of chloroform-soluble metabolites revealed that all the urine fractions contained similar fluorescent materials. Table 1 shows the number, fluorescence color, $R_F$ value, and proportion of each of the fluorescent compounds found in urine excreted up to 48 hr after dosing. All fluorescent compounds (except those in front of the

devolving solvent) were radioactive, and all radioactive compounds were fluorescent. Aflatoxin $M_1$ accounted for 2.3% of the dose, unmetabolized aflatoxin $B_1$ represented 0.01 to 0.1% of the dose, and the compound eventually identified as aflatoxin $P_1$ (7) represented about 1% of the dose in chloroform-soluble form.

**Water-soluble Metabolites.** Efficiency of the Amberlite XAD-2 column for extracting water-soluble metabolites from urine was such that 90 to 95% of radioactivity in the urinary aqueous phase was retained on the resin and subsequently eluted with methanol. Metabolites thus concentrated were dissolved in 0.2 M acetate buffer, pH 5.0, and subjected to enzymatic hydrolysis. Glucuronidase converted 58 to 60% of the radioactive material to a chloroform-soluble form, suggesting that a large proportion of metabolites was present as glucuronides. Treatment of the remaining aqueous residue with sulfatase converted a further 12% of the initial radioactivity to chloroform-soluble form, suggesting the presence of sulfate conjugates.

When the chloroform-soluble material from both enzymatic hydrolysies was subjected to thin-layer chromatography, both were found to contain primarily a yellow-green fluorescent compound with an $R_F$ value different from those of authentic aflatoxins $B_1$, $M_1$, and $B_2$. Autoradiography on X-ray film revealed a single radioactive spot that coincided with the fluorescent substance. On the basis of this evidence, it was concluded that conjugates cleaved by both enzymes contained the same derivative of aflatoxin $B_1$. This metabolite was later isolated and identified as aflatoxin $P_1$ (7).

Confirmation of results obtained by the enzymatic procedures was important for further characterization of the nature and quantitative relationships of urinary aflatoxin conjugates. A technique in which a DEAE-Sephadex A-25 column is used was developed for separation of unhydrolyzed aflatoxin conjugates, and Chart 2 shows the separation achieved for radioactivity in the urinary aqueous phase. Total radioactivity was separated into 6 subfractions. The major peak from this column contained 60% of the radioactivity, and subsequent enzymatic hydrolysis with the glucuronidase preparation suggested that 90% or more of the material in this subfraction was the glucuronide of aflatoxin $P_1$.

Thin-layer chromatograms of chloroform extracts of enzymatic incubation mixtures revealed contamination by other fluorescent compounds, and silica gel column chromatography was used to isolate and purify the major metabolite, aflatoxin $P_1$. Chart 3 shows the elution profile of such a column, in which 98% recovery was achieved. Two-dimensional thin-layer chromatography of the radioactive fraction, in which 2 different developing solvent systems were used (16), showed the absence of any contaminating fluorescent compound.

**Tissue Distribution of Radioactivity.** Radioactivity in various tissues was determined in animals killed at intervals after injection of aflatoxin $B_1$-$^{14}C$, and the main findings are summarized in Table 2. High initial values were found at 45 min; within 24 hr, radioactivity in all tissues reached a fairly constant level and declined slowly thereafter. Liver contained much greater amounts of radioactivity than any other organ at

![Chart 1. Cumulative urinary excretion of radioactivity from aflatoxin $B_1$-$^{14}C$ by 3 rhesus monkeys.](chart1)

**Table 1**

**Distribution of radioactive, chloroform-soluble urinary metabolites of aflatoxin $B_1$-$^{14}C$ on thin-layer chromatography**

<table>
<thead>
<tr>
<th>Position on chromatogram</th>
<th>Fluorescence color</th>
<th>$R_F$</th>
<th>Urine sample $b$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Origin</td>
<td>Yellow</td>
<td>0.00</td>
<td>26.8</td>
</tr>
<tr>
<td>Second</td>
<td>Blue</td>
<td>0.02</td>
<td>6.5</td>
</tr>
<tr>
<td>Third</td>
<td>Blue</td>
<td>0.05</td>
<td>3.5</td>
</tr>
<tr>
<td>$M_1$</td>
<td>Blue</td>
<td>0.14</td>
<td>31.6</td>
</tr>
<tr>
<td>$P_1$</td>
<td>Yellow-green</td>
<td>0.17</td>
<td>15.6</td>
</tr>
<tr>
<td>Sixth</td>
<td>Blue-green</td>
<td>0.23</td>
<td>1.4</td>
</tr>
<tr>
<td>Seventh</td>
<td>Blue</td>
<td>0.37</td>
<td>c</td>
</tr>
<tr>
<td>$B_1$</td>
<td>Blue</td>
<td>0.48</td>
<td>3.2</td>
</tr>
<tr>
<td>Ninth</td>
<td>Blue</td>
<td>0.55</td>
<td>0.5</td>
</tr>
</tbody>
</table>

$^{a}$ See Ref. 23 for chromatographic system used.

$^{b}$ Proportion of total radioactivity loaded on chromatogram in each fluorescent spot, determined by liquid scintillation counting of material recovered from plate. Each value is the mean of 4 to 6 samples.

$^{c}$ Amount of radioactivity present was less than detectable by liquid scintillation counting but clearly evident on autoradiograms.
J. I. Dalezios and G. N. Wogan

Chart 2. DEAE-Sephadex A-25 column chromatographic fractionation of radioactivity from the aqueous phase of urine of monkeys dosed with aflatoxin B$_1^{14}$C.

<table>
<thead>
<tr>
<th>ELUTANT</th>
</tr>
</thead>
<tbody>
<tr>
<td>A: ETHER-HEXANE (3:1)</td>
</tr>
<tr>
<td>B: 0.5% MeOH in CHC$_3$</td>
</tr>
<tr>
<td>C: 1.5% MeOH in CHC$_3$</td>
</tr>
<tr>
<td>D: 3.0% MeOH in CHC$_3$</td>
</tr>
</tbody>
</table>


DISCUSSION

Urinary Excretion Pattern. Within 24 hr after dosing, monkeys excreted in urine 26 to 30% of injected radioactivity all times. After 96 hr, the liver still retained 5.6% of the injected dose, more than 80 times the radioactivity contained in any other organ. The data in Table 2 show that radioactivity appeared in the small intestine, particularly in the duodenum, as early as 45 min after injection while, after 24 hr, radioactivity was found only in the ileum, reflecting ultimate excretion in feces. Early appearance of radioactivity in the small intestine suggested that aflatoxin B$_1$ and/or its metabolites are excreted rapidly in bile.

Distribution of Radioactivity in Blood. High levels of radioactivity were found in blood 10 min after the labeled toxin was injected, as illustrated in Table 3. A peak concentration was reached at 1 hr, and blood levels slowly declined thereafter. Radioactivity was principally contained in plasma throughout the study period, and within that compartment it was almost entirely associated with plasma proteins. Gel electrophoresis combined with autoradiography showed that the radioactivity in the plasma protein fraction was associated exclusively with albumin. Solvent extraction and thin-layer chromatography revealed that aflatoxin B$_1$ could be extracted from plasma during the 1st hr, but not thereafter.
Table 2

Tissue distribution of radioactivity from aflatoxin B1, 14C

<table>
<thead>
<tr>
<th>Organ</th>
<th>Radioactivity at following times after dosing</th>
<th>45 min</th>
<th>24 hr</th>
<th>48 hr</th>
<th>96 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Specific activity</td>
<td>% of dose</td>
<td>Specific activity</td>
<td>% of dose</td>
<td>Specific activity</td>
</tr>
<tr>
<td>Liver</td>
<td>21.2</td>
<td>19.1</td>
<td>8.3</td>
<td>8.3</td>
<td>6.9</td>
</tr>
<tr>
<td>Kidney</td>
<td>10.7</td>
<td>0.9</td>
<td>1.1</td>
<td>0.1</td>
<td>1.0</td>
</tr>
<tr>
<td>Adrenal</td>
<td>2.6</td>
<td>1.8</td>
<td>0.9</td>
<td>0.7</td>
<td>0.4</td>
</tr>
<tr>
<td>Testes</td>
<td>1.3</td>
<td>0.3</td>
<td>0.7</td>
<td>0.1</td>
<td>0.3</td>
</tr>
<tr>
<td>Heart</td>
<td>1.2</td>
<td>0.3</td>
<td>0.7</td>
<td>0.1</td>
<td>0.3</td>
</tr>
<tr>
<td>Pancreas</td>
<td>1.8</td>
<td>0.1</td>
<td>0.4</td>
<td>0.3</td>
<td>0.1</td>
</tr>
<tr>
<td>Spleen</td>
<td>1.3</td>
<td>0.1</td>
<td>0.5</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Thymus</td>
<td>1.0</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>0.5</td>
</tr>
<tr>
<td>Lung</td>
<td>1.0</td>
<td>1.0</td>
<td>0.4</td>
<td>0.4</td>
<td>0.4</td>
</tr>
<tr>
<td>Upper duodenum</td>
<td>45.0</td>
<td>0.3</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Lower duodenum</td>
<td>1.8</td>
<td>6.5</td>
<td>0.2</td>
<td>0.2</td>
<td>0.5</td>
</tr>
<tr>
<td>Diaphragm</td>
<td>1.2</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
<td>0.2</td>
</tr>
</tbody>
</table>

* dpm/mg wet organ; all values are means of 2 samples.

Table 3

Radioactivity in blood and fractions after i.p. injection of aflatoxin B1

One male rhesus monkey weighing 3.4 kg was given an injection of 10.6 X 10^4 dpm aflatoxin B1, 14C, and blood samples were taken at indicated times. Data are means of 3 samples.

<table>
<thead>
<tr>
<th>Fractions</th>
<th>Radioactivity at following times after dosing</th>
<th>10 min</th>
<th>1 hr</th>
<th>6 hr</th>
<th>24 hr</th>
<th>48 hr</th>
<th>72 hr</th>
<th>96 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole blood</td>
<td>5130</td>
<td>9394</td>
<td>8360</td>
<td>5130</td>
<td>4320</td>
<td>4146</td>
<td>3824</td>
<td></td>
</tr>
<tr>
<td>Plasma proteins</td>
<td>3934</td>
<td>7960</td>
<td>7322</td>
<td>4367</td>
<td>3700</td>
<td>3233</td>
<td>3162</td>
<td></td>
</tr>
<tr>
<td>Packed cells</td>
<td>2744</td>
<td>6273</td>
<td>7045</td>
<td>4260</td>
<td>3650</td>
<td>3203</td>
<td>3132</td>
<td></td>
</tr>
</tbody>
</table>

from labeled aflatoxin B1. The excretion rate reached a maximum within 3 hr, and about one-half of the radioactivity eventually excreted over a 4-day period was contained in the urine of the 1st 2 to 3 hr. In these respects, the pattern of excretion is, in general, similar to that of the rat (22). One important difference in the metabolism of aflatoxin B1 in the rat and monkey is the proportion of injected dose excreted as chloroform-soluble metabolites. In the monkey, only 15% of the radioactivity in urine was extractable with chloroform in contrast to rats, in which 50% of urinary radioactivity was chloroform soluble. The higher percentage of conjugated metabolites found in monkey urine might reflect a higher efficiency in conjugation in comparison with the rat. However, it might also be attributed to lower enzymatic activities of β-glucuronidase, sulfatase, and phosphatase found in monkey urine. Conzelman et al. (6) reported that the activities of these enzymes in monkey urine were lower than those in mice and dogs. In addition, they found that the urinary β-glucuronidase activity in the monkey was lower than that reported by Dryer et al. (9) for the rat. It is possible that the aflatoxin conjugates in rat urine might be partially hydrolyzed by the enzymes in the urinary bladder or following collection of excreted urine, and our results might have been influenced by this factor despite efforts to minimize it.

Metabolites of aflatoxin B1 identified in monkey urine were present mainly in water-soluble form, probably as conjugates of gluconic and sulfuric acids. Aflatoxin P1 represented the principal urinary aflatoxin derivatives, about 50% being present as glucuronide, 10% as sulfate, and 3% as unconjugated phenol. Together these represent more than 20% of the injected radioactivity from aflatoxin B1. By comparison, aflatoxin M1, the only other urinary metabolite of known structure, accounted for 2.3% of the administered dose, and unmetabolized aflatoxin B1 was found in much smaller quantities (0.01 to 0.1% of the dose). The urine contained also 3 additional unidentified metabolites within 1 hr after treatment and 1 [fluorescent Spot 7 (Table 1)] that was excreted only after the 1st 2-hr interval.

Since all metabolites separated by thin-layer chromatography were both radioactive and fluorescent, we conclude that there is little if any ring cleavage of aflatoxin B1 or at least that the chromophore moiety of the toxin molecule remains essentially intact. This was also the case in the rat, in which all chloroform-soluble urinary metabolites of aflatoxin
B1 were both radioactive and fluorescent (J. Dalezios and G. N. Wogan, unpublished data). Aflatoxin P1 represents a large fraction of urinary derivatives, and the reason that the compound was not found earlier is probably that only a small fraction of the total amount in urine is present in unconjugated, chloroform-soluble form (1% of dose). Previous investigators have concerned themselves almost exclusively with the fraction containing chloroform-soluble materials, and this probably accounts for the fact that the metabolite was not detected earlier.

If it can be established that aflatoxin P1 is a urinary metabolite of aflatoxin B1 in man and that its excretion is quantitatively related to aflatoxin B1 intake, then exposure of human populations to the toxin could be estimated by screening of urine samples (7). This approach has already been attempted with aflatoxin M1 levels in urine used as the index of ingestion (4), but low levels of recovery prohibited quantitative estimation of toxin intake. If man excretes aflatoxin P1 in quantities comparable to those of the monkey, it may be feasible to utilize this approach in epidemiological surveys.

Radioactive compounds derived from aflatoxin B1,14C rapidly appeared in all tissues examined, and maximum specific activity was attained 45 min after administration. After 96 hr, specific activities of all tissues other than liver and kidney had declined to very low values. During the entire 4-day period, the liver contained high amounts of radioactivity, and at the end of this period it still retained 5.6% of the administered dose. These findings are in general agreement with those for the rat, reported earlier (22). Persistence of radioactivity in rat liver (5.6% of the dose at 4 days) was also observed by Lijinsky et al. (14). In both cases, most of the liver radioactivity was associated with the total protein fraction. Liver, which possesses the metabolic apparatus for drug metabolism, retains more radioactivity from aflatoxin B1 for longer periods of time than any other tissue. This might be associated with formation of a metabolic product(s) that is very reactive with cellular macromolecules.

Many in vitro studies have dealt with the interaction of aflatoxins with cellular macromolecules, especially DNA (5, 17, 23). Interactions of aflatoxins with cellular macromolecules, under in vivo conditions, have been studied in only 1 earlier series of experiments (14). Injection of tritiated aflatoxin B1 into rats resulted in incorporation of radioactivity into liver RNA, DNA, and protein; the protein fraction was the most highly labeled. The present experiments with monkeys also demonstrated the association of radioactivity derived from aflatoxin B1,14C with proteins.

Distribution of radioactivity derived from aflatoxin B1,14C in blood fractions, including plasma proteins, has not been previously reported. In these experiments, 1 hr after dosing, 20% of blood radioactivity was extractable with solvents while, after 24 hr, practically all radioactivity was associated with the plasma proteins. Thin-layer chromatography of the extractable radioactivity revealed that the chloroform-soluble material was mainly aflatoxin B1. These data do not provide direct evidence of the nature of the radioactive material interacting with plasma proteins. However, the sharp decrease in unbound radioactivity in blood plasma seen to occur after the 1st hr suggests strong binding rather than a reversible interaction.

Gel electrophoresis of blood serum proteins revealed that the radioactivity was associated exclusively with the albumin fraction of serum. Although the biological significance of aflatoxin interaction with plasma albumins is unknown, there is indirect evidence that steroids bound to globulins and albumins are physiologically inactive (13). A strong affinity of aflatoxins for albumins, expressed by the formation of a covalent bond, would decrease the concentration of the available toxin at the target organ and thus indirectly affect responses to it.

REFERENCES

Aflatoxin Metabolism in Monkeys


Metabolism of Aflatoxin B₁ in Rhesus Monkeys

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