THE METABOLISM OF AFLATOXIN B₁ IN RATS

I. F. H. PURCHASE and M. STEYN

From the Division of Toxicology, National Nutrition Research Institute of the Council for Scientific and Industrial Research, Pretoria, South Africa

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Aflatoxin B₁, a carcinogenic metabolite of the fungus Aspergillus flavus, is acutely toxic to a number of species, including rats. Male rats (LD₅₀ 7.2 mg./kg.) are more susceptible to the acute oral effects of aflatoxin than are females (LD₅₀ 17.9 mg./kg.) (Butler, 1964).

Aflatoxin B₁ is metabolised to aflatoxin M₁ which is present in the blood and urine of dosed rats (Butler and Clifford, 1965) and in the milk of lactating rats, sheep and cows ingesting aflatoxin (de Jongh et al., 1964a; Nabney et al., 1967). Aflatoxin M₁ is the hydroxylated derivative of aflatoxin B₁ (Holzapfel et al., 1966) and has the same acute toxicity as aflatoxin B₁ in ducklings (Purchase, 1967). The presence of aflatoxin M₁ in the blood indicates that this compound is a metabolic product of aflatoxin B₁. The difference in the susceptibility of male and female rats to aflatoxin may be a result of a difference in the ability of males and females to metabolise aflatoxin. This report describes certain quantitative aspects of aflatoxin metabolism in rats.

METHODS

Analytical method

Previous methods for aflatoxin analysis in tissues have been qualitative (e.g. de Jongh et al., 1964b). It was necessary to have a method which provided consistent results for quantitative assessment of aflatoxin metabolism. Six female Wistar rats (approximately 200 g. each) of our own strain were dosed with aflatoxin B₁ in dimethylsulphoxide (DMSO) per os at a dose of 10 mg./kg. body weight. The rats were killed with ether 40 minutes later and the stomachs, with contents, livers and kidneys were removed. The stomachs from all 6 rats were pooled and homogenised in water with a blender at 3000 r.p.m. until a smooth homogenate was obtained. The livers and kidneys were homogenised together in a similar way. Aliquots of known weights from the two homogenates were homogenised at 3000 r.p.m. for 1 minute with various solvents (methanol or acetone or an azeotropic mixture of acetone, chloroform and water 38 : 58 : 4). The homogenates were filtered, the residue rinsed and the filtrate evaporated to dryness under reduced pressure.

The quantity of aflatoxin in each extract was determined after chromatography on silica gel (Camag D-5) thin layer chromatoplates with a Photovolt Model 530 densitometer using the method described by Pons et al. (1966). Appropriate solutions of pure aflatoxin M₁ and B₁ were used as standards.
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Aflatoxin metabolism

Adult male (mean weight 359 g.) and adult female (mean weight 220 g.) rats were distributed into 7 groups, each containing 3 males and 3 females. Each rat was housed in a separate cage and received an oral dose of aflatoxin B₁ (10 mg./kg.) dissolved in DMSO (10 mg./ml.). The sample of aflatoxin B₁ was chromato-graphically pure and contained 98% aflatoxin B₁ on assay by u.v. absorption spectrophotometry. Groups of rats were killed with ether at ½, 1, 2, 4, 6 and 8 hours after dosing and their livers, kidneys, stomachs and intestines removed and placed in separate weighed containers. The organs were stored at -17° C. until assayed for aflatoxin B₁ and M₁. One group of rats was dosed with DMSO (1 ml./kg.) and killed at 2 hours and the organs removed for analysis.

Each sample consisting of one liver, or two kidneys or the stomach and intestines of one rat was homogenised with 10 ml. of acetone : chloroform : water (38 : 58 : 4) azeotrope at 3000 r.p.m. for 1 minute. The homogenate was filtered, the filtrate dried under reduced pressure and the residue redissolved in a known quantity of benzene. The quantity of aflatoxin B₁ and M₁ was assayed as described above and the final result expressed as µg./organ or µg./g. organ. The results are expressed as an average of the 3 rats of the same sex in each group.

RESULTS

Analytical method

The azeotropic mixture of acetone, chloroform and water extracted more aflatoxin B₁ from the stomachs than did methanol and provided more consistent recoveries (Table I). Although the azeotrope did not extract more aflatoxin B₁ and M₁ from the liver and kidney homogenate than did methanol or acetone, the quantities recovered were more constant than those recovered with the other solvents. The azeotropic mixture, which has previously been shown to be most effective for extracting aflatoxin M₁ from milk (Purchase and Steyn, 1967), was selected as the solvent of choice for this study on the basis of the more consistent recoveries.

| Organ     | Solvent         | Mean (µg./g.) | SD | CV  | Mean (µg./g.) | SD | CV  |
|-----------|-----------------|---------------|----|-----|---------------|----|-----|
| Stomach   | Methanol        | 17.25         | 2.50 | 14.5 |               |    |     |
|           | Azeotrope       | 20.28         | 1.45 | 7.2  |               |    |     |
| Liver     | Methanol        | 4.04          | 1.80 | 45   | 1.09          | 0.52 | 48  |
|           | Acetone         | 3.21          | 0.55 | 17   | 1.49          | 0.53 | 36  |
| Kidney    | Azeotrope       | 3.14          | 0.37 | 11   | 1.45          | 0.16 | 11  |

SD = standard deviation.  
CV = coefficient of variation.

TABLE I.—Results of Extraction of Tissue Homogenates with Various Solvents. Four Aliquots were Extracted with Each Solvent

Aflatoxin B₁ metabolism

The average weights of the liver, kidney and intestines remained constant over the 8 hour experimental period. Results of assay of livers and kidneys are thus expressed as µg./g. organ but those on the intestine are expressed as µg./organ.
Aflatoxin B₁ content of various organs.—The aflatoxin B₁ content of the stomach and intestines decreased over the experimental period until about 20% was recoverable at 8 hours (Fig. 1). There was no consistent difference between males and females. A further experiment in which the stomachs and intestines were assayed separately showed that relatively little (<10 μg. or 0.005%) of the total dose of aflatoxin B₁ was present in the kidneys and liver.

Aflatoxin M₁ content of various organs.—The highest concentration of aflatoxin M₁ occurred in the kidneys and the concentrations were consistently higher in the females than in the males (Fig. 2). The increase in the concentration of aflatoxin M₁ at 8 hours in the females was due to an extremely high value (3.2 μg./g.) in one animal and may be due to a technical error.
A similar trend was observed in the livers although the concentrations did not reach such high levels (Fig. 3). The aflatoxin M₁ concentrations in the stomach and intestines were variable, but the figures for the females were consistently higher than for the males (Fig. 4). In a separate experiment where the stomach and intestines were assayed separately, it was found that the aflatoxin M₁ was present in the intestines only.

![Graph showing concentration of aflatoxin M₁ in the liver of rats given a single dose of aflatoxin B₁ (10 mg./kg.).](image)

**Fig. 3.**—The concentration of aflatoxin M₁ in the liver of rats given a single dose of aflatoxin B₁ (10 mg./kg.).

![Graph showing total quantity of aflatoxin M₁ recovered from the stomach and intestines of rats receiving aflatoxin B₁ (10 mg./kg.).](image)

**Fig. 4.**—The total quantity of aflatoxin M₁ recovered from the stomach and intestines of rats receiving aflatoxin B₁ (10 mg./kg.).

**Other fluorescent metabolites**

Traces of fluorescent metabolites were observed in some extracts. Aflatoxin B₂ₐ (Dutton and Heathcote, 1966) was recovered from the stomachs of most animals.
Control rats

No aflatoxin was observed in the extracts from the control rats.

DISCUSSION

The amount of aflatoxin absorbed from the stomach was similar in males and females over the 8 hour period of the experiment and therefore the difference in susceptibility of males and females cannot be explained on the basis of differing rates of absorption. The concentrations of aflatoxin M₁ in the organs of female rats were considerably higher than those in males and detectable levels were observed for much longer periods. This indicates that the metabolic pathways responsible for converting aflatoxin B₁ into M₁ are more active in females than males. The reason for the greater susceptibility of males may be, therefore, that the aflatoxin B₁ is detoxified more slowly in males than in females. The presence in females of a higher concentration of aflatoxin M₁, which has the same acute toxicity in ducklings as aflatoxin B₁ (Purchase, 1967), probably does not contribute significantly to the toxic effect as there is only a very small percentage (<0.1%) of the initial dose of aflatoxin B₁ present as aflatoxin M₁ at a given time.

The metabolic pathway responsible for converting aflatoxin B₁ to M₁ is presumably the "drug-metabolising enzymes" because it is present in the microsomal fraction of liver cells (Schabort, 1969). The reason for the higher activity in females may be a differential action of the male and female hormones on this enzyme system. This could be due to a non-specific stimulation of hydroxylating enzymes by progesterone or oestrogens in the female or alternatively inhibition by testosterone. Stimulation by female hormones would seem to be the more likely alternative as the non-specific stimulation of the enzymes by, for example, barbiturate-rates increases the hydroxylation of aflatoxin B₁ in rat liver (Schabort, 1969).

The presence of measurable quantities of aflatoxin M₁ in the intestine indicates that this compound is excreted in the bile and may have an entero-hepatic circulation. Whether aflatoxin M₁ is a minor metabolite or an intermediate in a major metabolic pathway cannot be deduced from these results.

SUMMARY

Aflatoxin B₁, dissolved in dimethyl sulphoxide, was dosed orally to groups of male and female rats. The amount of aflatoxin B₁ and aflatoxin M₁, a fluorescent metabolite, was determined in liver, kidneys, stomach and intestine at intervals after dosing. There was no difference between the rate of uptake of aflatoxin B₁ from the stomachs of male and female rats. There was, however, more aflatoxin M₁ in the liver, kidney and intestine of female rats, suggesting that the female rat is capable of metabolising aflatoxin B₁ at a faster rate than males. This may explain the greater resistance of females to the acute toxic effects of aflatoxin B₁.

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