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Urinary excretion of aflatoxin M_1 after administration of aflatoxin B_1 in sucrose- or starch-rich diets

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1. Male Sprague–Dawley rats were given 630 g/kg sucrose or starch with 2 mg/kg aflatoxin B_1 for periods of 75, 145 and 200 d, and the 24 h urinary excretion of aflatoxin M_1 was measured.

2. Less aflatoxin M_1 was excreted by the rats fed on the sucrose-rich diet compared to those fed on the starch-rich diet. This difference was especially marked when expressed per g metabolizing tissue.

3. It is concluded that sucrose probably decreases the activity of aflatoxin B_1 metabolism in a similar way to its previously found effect on the drug-metabolizing enzyme.

Aflatoxin B_1 is produced by the common food-spoilage fungus *Aspergillus flavus*, and it is both the most frequently found and the most potent carcinogen of this group of compounds (Wogan, 1973). There is also some epidemiological evidence of its carcinogenicity in man (Campbell & Stoloff, 1974). Hydroxylation of aflatoxin B_1 to aflatoxin M_1 occurs in the drug-metabolizing complex of the liver (Campbell & Hayes, 1974). Aflatoxin M_1 has been found in human urine (Campbell, Caedo, Bulatao-Jayme, Salamat & Engel, 1970; Maleki, Suzangar, Jamshidi, Tahasebi & Barnett, 1977) and might be a useful indicator of aflatoxin B_1 consumption.

Many nutrients are required for the proper functioning of the drug-metabolizing complex (Campbell & Hayes, 1974), but there are no known studies of effects of nutrition on aflatoxin B_1 metabolism. It has been established for some time that carbohydrate decreases the activity of drug-metabolizing enzymes (Strother, Throckmorton & Herzer, 1971) and some in vitro experiments demonstrated that sucrose has a greater effect in decreasing these enzymes than does starch (Basu, Dickerson & Parke, 1975). These authors demonstrated decreased levels of biphenyl 2-hydroxylase and biphenyl 4-hydroxylase of liver microsomes. At the same time they found lower cytochrome P-450 levels. In vivo studies have shown that sucrose increases sleeping times after hexobarbital (Basu *et al.* 1975) and increased toxicity of benzylpenicillin (Boyd, Dobos & Taylor, 1970). In the present report, the urinary excretion of aflatoxin M_1 was measured during a period of 200 d feeding with aflatoxin B_1 in sucrose- or starch-rich diets. This work is part of a series of projects designed to investigate the variations of aflatoxin M_1 excretion caused by dietary factors which might be important in the use of this factor as an estimator of aflatoxin B_1 consumption.

METHODS

Male rats were bred from parents originally obtained from the Sprague–Dawley colony at the Pasteur Institute, Tehran. At 4 weeks of age, they were divided into two diet groups of approximately equal average weights (40 g) and were given 2 mg/kg aflatoxin B_1 (Makor Chemicals Ltd, Israel) in either sucrose- or starch-rich diets. The diets contained (g/kg): casein 250, maize oil 50, mineral mix (Porter, 1963) 60, vitamin mix (Porter, 1963) 10, and

Table 1. *The weights of rats and their 24 h urinary aflatoxin M_1 excretion rates after receiving 2 mg aflatoxin B_1 kg in sucrose- or starch-rich diets*

(Mean values and standard deviations)

Diet ...		Starch					Sucrose				
					Aflatoxin M_1 excretion					Aflatoxin M_1 excretion	
Period of Experiment (d)		Wt (g) Mean	SD	No. of rats	μg/kg per d Mean	SD	μg/kg per g liver per d Mean	SD	Wt (g) Mean	SD	No. of rats	μg/kg per d Mean	SD	μg/kg per g liver per d Mean	SD
75	Mean	115	24	5	3·1	3·6	870	1200	128	25	5	1·4	0·92	200	140
	Median				1·7		370					1·3		160	
145	Mean	134	40	8	2·1	2·3	490	194	156	32	9	2·0	1·4	350	220
	Median				1·9		430					1·4		300	
200	Mean	180	42	3	1·8	0·4	260	36	164	19	3	1·2	0·82	150	59
	Median				1·8		260					1·1		140	
Totals															
	Mean			16	2·3	2·0	560	660			17	1·7	1·2	270	260
	Median				1·8		370					1·4		190	
	Ranges				1·0–9·5		230–2900					0·5–4·7		60–940	

either starch 630 or sucrose 630. Because of the hazardous nature of the separation of faeces and spilt food, the food intake was only determined once at 140 d of experiment to show whether there were gross differences between the intakes of the two groups. The experiment was terminated for one group after 75 d, two groups after 145 d, and the remaining group after 200 d. During the last week of life, urine was collected, and then the rats were killed and the liver dissected and weighed. The livers were examined microscopically and the progress to liver damage was assessed by reference to the histopathological studies of Newberne & Wogan (1968) on aflatoxin B_1-induced carcinomas.

Urine was collected from each rat over a period of 24 h, beginning at 08.00 hours. During urine collection, water was freely available but food was not given while the rat was in the metabolism cage. Urine dripped into a beaker containing 10 ml chloroform and 10 ml 0·09 M-saline. The aflatoxin M_1 was extracted into the chloroform by gentle swirling, and the aqueous fraction was extracted with a further volume of chloroform. The extract was dried with anhydrous sodium sulphate and evaporated to dryness under nitrogen. The sample was then redissolved in 100 μl chloroform and was analysed by thin-layer chromatography (Association of Official Analytical Chemists, 1975) and confirmed as aflatoxin M_1 by the chemical method of Trucksess (1976).

RESULTS AND DISCUSSION

There was no significant difference in the growth of sucrose-fed and starch-fed rats, and this is reflected in the similarities of food intake at 140 d of experiment: 61 g/kg for starch-fed rats compared to 64 g/kg for sucrose-fed rats. Therefore, on average, at that age, the daily intake of aflatoxin B_1 was 122 μg and 128 μg respectively. During the experiment, only three rats died, although the growth rate was approximately 10–15% less than usually found in this rat colony when given the same diet without added aflatoxin B_1. Table 1 shows that the excretion of aflatoxin M_1 (μg/kg per d) was less in sucrose-fed rats. Although most of the values were very similar, certain rats excreted much more than average amounts of aflatoxin M_1, and thus the analyses were duplicated. Because of this biological variation, the standard deviations were large and the mean values were higher than the medians, indicating a non-normal distribution. Therefore, the Mann–Whitney U test was used to test for significance of the different distributions of the aflatoxin M_1 excretions of the two groups. The medians and ranges of values are presented as well as mean values and standard deviations. Although the difference between the two groups was not large, it was statistically significant ($P < 0{\cdot}05$). The livers of the sucrose-fed rats were a larger proportion of the body weight (49·5 ± 7·5 g/kg) than in the starch-fed rats (39·1 ± 7·5 k/kg). Therefore the sucrose-fed rats had a larger quantity of tissue available for metabolism. The results were therefore also expressed on a per g liver basis, indicating that the sucrose-fed rats excreted almost half as much aflatoxin M_1 and this was a very significant difference ($P < 0{\cdot}005$)

Previous work on labelled aflatoxin B_1 metabolism showed no difference in excretion between oral and intraperitoneal administration (Wogan, Edwards & Shank, 1967). During the 24 h period after peritoneal injection, 25% of the total radioactivity was found in the urine. When labelled in the methoxy position, only 22% of radioactivity appeared in the faeces, but when ring-labelled, 57% was excreted in the faeces. Because very little radioactivity remained in the body it would appear that the 24 h collection period chosen for this present study was adequate to collect most of the aflatoxin B_1 administered on the previous day. The recovery of aflatoxin M_1 in urine has been given as 1–4% (Campbell *et al.* 1970), and 2·3% (Dalezios, Wogan & Weinreb, 1971), and in the present study the majority of values were 1–3%.

Table 2. *The mean degree of liver damage (scale 0–3) as seen in rats fed aflatoxin B_1 in diets containing sucrose or starch*

Period of experiment (d)...	75		145		200	
Diet...	Sucrose	Starch	Sucrose	Starch	Sucrose	Starch
No. of rats	5	5	9	8	3	3
Lymphocytic infiltration	1	0	1·5	0·5	0·3	1·0
Degeneration	2·2	0	1·0	1·0	1·0	1·3
Fibrosis	1	0·5	1·6	0·1	2·3	2·0
Necrosis	0·6	0·5	0·6	0·3	2·3	1·3
Hyperplasia	0	0	0·7	0	2·3	0
Tumours	0	0	0	0	0	0

The liver slices have been graded according to the degree of damage seen (0–3), when compared to controls that were not given aflatoxin B_1 (Table 2). No histological difference was found between sucrose- and starch-fed controls. It is impossible to statistically verify the slightly greater tendency shown for more hyperplasia in the older sucrose-fed rats. From this data it is not obvious whether the degree of liver damage is an important determinant of aflatoxin M_1 excretion, but it should be pointed out that the difference in excretion data between sucrose- and starch-fed rats appeared also in both the younger age groups, when no difference in histology was evident.

In conclusion, aflatoxin M_1 excretion was lower in sucrose-fed rats, especially when expressed per g metabolizing tissue. This is probably due to the decreased activity of the drug-metabolizing enzyme previously found in sucrose-fed rats, although it could be attributed to other unmeasured factors such as absorption rate, blood transport, or kidney function. However, the possible importance of sucrose substitution in human diets on drug metabolism is a subject worthy of further study. Also the agreement between the results on aflatoxin M_1 excretion and previous studies on drug metabolism suggest that further work should be undertaken to study aflatoxin M_1 excretion after ingestion of other diets known to influence drug metabolism. The recent discovery of aflatoxin P_1 in urine (Campbell & Hayes, 1974) opens an even wider field of study of nutritional effects on aflatoxin B_1 metabolism and its possible importance in carcinogenesis.

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