The effects of gum arabic, wheat offal and various of its fractions on the metabolism of ^{14}C-labelled aflatoxin B_1 in the male weanling rat

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1. Male weanling rats were given for extended periods diets containing gum arabic or wheat offal or various offal fractions. The fractions included two lipid fractions, a water-soluble extract and a modified-acid-detergent (MAD)-fibre extract. The diets contained either low concentrations of aflatoxin B_1 (induced rats) or were free from this source of aflatoxin (non-induced rats). The distribution of ^{14}C was then studied after the rats received ^{14}C-labelled aflatoxin B_1 in their feed. Blood plasma concentrations of triglycerides, and total cholesterol were also measured.

2. Gum arabic and wheat offal accelerated the rate of passage of ^{14}C through the small intestine and wheat offal very considerably decreased retention time in the large intestine. Both fibre sources increased faecal bulk. However, only wheat offal decreased liver and urinary accumulation of ^{14}C and the effect could be explained entirely by the MAD-fibre fraction of wheat offal.

3. The possible induction of either microsomal enzymes unrelated to the production of mutagenic aflatoxin metabolites, or of extramicrosomal enzymes is discussed; but it is concluded that the main effect brought about by wheat offal on the toxicity and carcinogenicity of aflatoxin can be attributed to a direct influence of the MAD fibre fraction of wheat offal on the intestinal absorption of aflatoxin B_1.

4. The relevance of these conclusions to drug safety studies is discussed, because comparable studies may yield differing results, despite a use of diets having the same nutrient composition but differing ingredient composition.

A previous investigation demonstrated that the faecal excretion of ^{14}C by rats given ^{14}C-labelled aflatoxin B_1 orally was increased when a part of their dietary starch was replaced by wheat offal (Frape *et al.* 1981). There are several mechanisms by which such an effect might be achieved.

It has been demonstrated by a number of workers that wheat bran at a dietary concentration of approximately 500 g/kg reduces cholesterol and total lipids in the livers of rats given diets containing cholesterol (Ranhotra, 1973; Kay & Truswell, 1975; Truswell, 1975; Yacowitz *et al.* 1976), that it influences bile secretion and absorption (Riottot *et al.* 1975; Wicks *et al.* 1978) and, by binding bile acids and cholesterol to its fibre fraction, increases their faecal loss (Owen *et al.* 1975; Eastwood & Mowbray, 1976; Yacowitz *et al.* 1976; Ranhotra *et al.* 1977). Bile secretion and micelle formation play important roles in the intestinal absorption of fat-soluble dietary constituents and therefore the absorption and metabolism of fat-soluble toxins may be modified by a change in enterohepatic circulation. Furthermore it has been suggested, but little evidence has been advanced, that toxins in the digesta may be adsorbed to dietary fibre and voided in the faeces.

Several dietary factors, including protein (Hayes *et al.* 1978) affect the activity of the hepatic microsomal mixed function oxidase system and thus influence the metabolism of chemical carcinogens (Wattenberg, 1975).

In two of four experiments previously reported (Frape *et al.* 1981) the intestinal absorption, retention and excretion of ^{14}C-labelled aflatoxin was measured over a period of several days following a single dose in rats receiving wheat offal or gum arabic in their diet. The purpose of the experiments to be described was to investigate the distribution of ^{14}C radioactivity in male rats relatively soon after an oral dose of ^{14}C-labelled aflatoxin B_1 and to measure how this distribution might be influenced by replacing a portion of the dietary starch by wheat offal or gum arabic. Secondly it was the purpose to obtain some indication of the factors which might be operating in wheat offal to effect the changes observed.

EXPERIMENTAL

Expt 1

Six male weanling rats (MRC grade 4 Charles River strain) in two litter groups of three rats each were caged individually on screened-floors, given free access to water from nipple drinkers and given a dry powdered diet at a rate of 8 g/rat daily. In each group two rats received diet J and one received diet K in which the polysaccharide sources were maize starch (J) and gum arabic (K) (Frape *et al.* 1981). After 8 weeks tail cups were fitted (Frape *et al.* 1970) and the rats were deprived of dry feed for 1 d. On the following day each rat received 3 g feed containing 0·22 μCi ^{14}C-labelled aflatoxin B_1 (Makor Chemicals Jerusalem). In each group the rat on diet K and one of those on diet J were given the tracer in the diet they had been receiving previously. The other rat on diet J, was given its tracer dose in diet K. All rats consumed their feed allowance within 20–30 min from time of access. Urine was collected after 45 min from time of access in 80 ml 1 M-acetic acid and all faecal pellets were collected in the tail cups. One group of three rats was killed with nitrogen gas 5·0 h from time of access to the feed and the other at 16·2 h from time of access.

At death a sample of blood was aspirated from the posterior vena cava and the plasma separated for solution in NCS (Amersham/Searle Corp). The gastrointestinal tract was removed and divided into its several functional sections with the contents left *in situ* in preparation for freeze-drying and oxidation in a Packard 305 Oxidizer. Kidney and liver samples were retained for histological examination and for solution in NCS. A portion of the urine was extracted with chloroform–methanol (95:5, v/v) for thin-layer chromatography on silica gel and autoradiography and the remainder was digested in PCS (Amersham/Searle Corp). Measurements of ^{14}C radioactivity were made in a Beckmann liquid-scintillation counter and quenching was assessed by internal spiking with ^{14}C-labelled hexadecane. The results were expressed as total activity in the organ, tissue, urine and region of gastrointestinal tract plus contents, as a percentage of the total recovered per rat at death.

The previously-described design was repeated on five further occasions, with the exception that on the last three all rats (induced) received mixed aflatoxins providing 1 mg aflatoxin B_1/kg diet during the last 12 d of the experiment. A separate statistical analysis was carried out on the values collected from each of the two time periods, 0–5·0 h and 0–16·2 h (time of access until death). Thus the experiment consisted of a 3 × 2 factorial arrangement of three dietary treatments given to induced and non-induced rats, with blocking by litter within each induction group.

Expt 2

Twenty-four male weanling F_1 cross-bred rats (Albino × Hooded, Spillers strains, cross-bred) were allotted to cages as in Expt 1. Six replicates of four rats having similar initial weights were formed and four diets were allocated at random to each replicate. The diets were of the same basal composition as that used in the previous experiment. The polysaccharide content of diet A was composed of 344 g maize starch/kg diet. In diet B 300 g maize starch

was replaced by wheat offal. In diets H and L small proportions of the starch were replaced by a chloroform–ethanol–water (8:6:1, v/v) extract of 300 g wheat offal (diet H) and by a diethyl ether extract of 300 g wheat offal (diet L). These diets were given restrictedly and daily in equal weights to each rat for 6 weeks. During the last 10 d all rats were induced and dosed with ^{14}C-labelled aflatoxin by the method used in Expt 1 except that the dose was 0·8 μCi ^{14}C-labelled aflatoxin B_1 (Moravek Biochemicals, City of Industry Ca-USA). All feed was consumed by 20–30 min after access to the meal. Tail cups were not fitted but urine and faeces were collected and the first replicate was killed 1·45 h from the time of access to the meal and the last replicate after a further 6·60 h. One additional rat allotted to each diet was killed 40·7 h after access to the meal. Where not stated, procedures were similar to those described for Expt 1, with the exception that each functional region of the gastrointestinal tract with its contents was freeze-dried, weighed and blended. Duplicate samples were then taken for oxidation. In addition a sample of thigh muscle (approximately 0·08 g dry matter (DM)) was removed from each of the rats, sampled, freeze-dried and digested in NCS for counting. Values are given in Table 3 as disintegrations/min per entire organ, urine excreted, or in calculated total skeletal muscle mass (where this mass = carcass weight × 0·5, and where carcass weight = body-weight less weights of gastrointestinal tract, pancreas, liver, kidneys and approximately 2 ml blood).

Expt 3

Seven male weanling F_1 cross-bred rats (Albino × Hooded, Spillers inbred strains, cross-bred) were allocated to each of the diets A, B, C, D, E, G and H in randomized blocks and caged as before (group 1). A further eight rats were allocated similarly to each of the diets C, D, E, G and H only (group 2). The basal diet was of the same composition as that used in Expt 2 (diet A). Diets B and D were formed by replacing 300 g maize starch by 300 g raw wheat offal/kg diet. Diet C had the same ingredient composition as diet A. Diet E contained 300 g autoclaved ($1·056 \times 10^4$ kg/m^2 for 30 min) wheat offal in place of the raw wheat offal. Diet G was the same as C but a water extract of 300 g wheat offal/kg was added replacing an equal weight of starch and in diet H a chloroform–ethanol–water (8:6:1, v/v) extract of 300 g wheat offal replaced an equal weight of starch. Diets C, D, E, G and H also contained 2 mg aflatoxin B_1/kg (Makor Chemicals Ltd) from the start of the experiment, whereas diets A and B were free of aflatoxin. After 15 weeks one rat on each of treatments A and B (both from group 1) and seven on each of treatments C, D and H (three from group 1 and four from group 2 in each case) were placed in twenty-three metabolism cages and given in their feed ^{14}C-labelled aflatoxin B_1 following the procedure outlined for Expt 2 with the exception that the urine and faeces were divided into three consecutive periods of excretion. For the urine these were 0–19 h, 19–48 h and 48–168 h respectively after access to the feed, and for faeces they were 0–48 h, 48–96 h and 96–168 h respectively.

The rats were killed in blocks 7, 8 and 10 d after dosing when the liver ^{14}C radioactivity was measured (Table 5) and the blood plasma was analysed (Table 6). Rats not used for the tracer study were killed on the same day as their tracer 'block'-mates, having received the same quantity of food, and the values were incorporated in the values given in Tables 4 and 6. Postmortem, the procedure adopted, was that described in Expt 2. Blood lipid analysis was restricted to the seven blocks from which the tracer rats were taken and four other blocks.

The values of groups 1 and 2 were analysed separately in Tables 4 and 6 because the plasma lipid values were determined on different instruments, and an earlier comparison had indicated a scaling difference which was not simply a shift of origin. The carcass and organ weights in Table 4 are given separately for the two groups so that direct comparisons can be made with Table 6.

Expt 4

Twenty-four male weanling F_1 cross-bred rats (Albino × Hooded, Spillers inbred strains, cross-bred) were allocated to metabolism cages and managed as in Expts 2 and 3. Six replicates of four rats each were formed and four diets (B, C, D and E, see below) were allocated at random to each replicate. The diets were fed restrictedly at a rate of 8 g rising to 9·5 g/rat daily over a period of 32 d. After this, the feed allowance for 1 d was omitted and on the following day each rat was given 3 g of its diet to which 0·8 μCi ^{14}C-labelled aflatoxin B_1 had been added. Three of the replicates received their feed at 08.05 hours and three at 09.05 hours. Urine was collected and the rats were killed in replicates in such a way that one replicate from each feeding time was killed at each of three different times, 5·0, 5·3 and 5·7 h, after access to feed. At death, blood, livers, gastrointestinal tracts, including contents, and samples of thigh muscle were removed and together with the urine treated as described in Expts 2 and 3, with the exception that after freeze-drying, samples of liver and muscle were digested in Soluene-350 (Packard Instrument Co), and analysis of the gastrointestinal tract was restricted to stomach and large intestine constituting two functional regions.

Suit *et al.* (1977) demonstrated that dietary differences could influence the metabolism of aflatoxin B_1 by rat hepatic microsomal fractions. Thus a further study was conducted. Twenty-four F_1, cross-bred male weanling rats were allocated in four replicates of six rats to five diets (A, B, C, D and E) so that within a replicate two rats received diet A and one rat each of diets B, C, D and E. The ingredient composition of diets A and B was the same as the basal diet A used in Expt 2. Diet C contained 300 g wheat offal as found in diet B of Expt 2. Diet E contained a chloroform–ethanol–water extract as used in diet H of Expt 2 and diet D contained 48·6 g of a modified-acid-detergent (MAD)-fibre extract. (This was extracted by a modification of the method of Van Soest (1963) in which 20 g were refluxed for 2 h in 1 l 0·5 M-sulphuric acid containing 10 g cetyl trimethyl ammonium bromide. The residue was recovered by filtration and was washed with distilled water to attain pH 7. The residue was then dried by washing with acetone and heating to 60°.) The extract came from approximately 300 g wheat offal and replaced the same amount of starch. Aflatoxin B_1 (0·5 mg/kg diet) was included in diets B, C, D and E from the start of the experiment. The rats were caged individually on screened-floors at the time the first twenty-four were allocated to treatment and they were given feed at a similar rate for 43 d. Following a 24 h fast they were killed, when blood and liver samples were retained. The liver samples were homogenized at 0° in a Potter-Elvehjem apparatus. The homogenate was centrifuged at 9000 ***g*** and the S-9 fraction harvested and stored as 2 ml portions in liquid N_2. The latter portions were used in a bacterial test for mutagens described by Ames *et al.* (1975). In this test, cultures of *S. typhimurium* TA98 and TA100 were subjected to aflatoxin B_1 and activated by the microsomal S-9 fractions. These fractions included all treatments A, B, C, D and E and therefore came from aflatoxin-induced and non-induced rats. Sufficient material for the test could only be obtained by combining the portions from rats on the same treatment.

RESULTS

Expt 1

Comparisons amongst diets showed that the specific activity and total activity in the blood plasma was greater in rats receiving diets JK or KK than in rats receiving diet JJ at 5·0 h ($P < 0·01$), but at 16·2 h no difference was found (Table 1). In the period 0–5·0 h total faecal ^{14}C losses did not seem to differ between treatments, whereas over 16·2 h the total ^{14}C in the faeces of rats on diet KK appeared to be greater than that in the other two groups.

Table 1. *Expt 1. Treatment mean total ^{14}C radioactivity estimated for organ, ingesta and plasma at time of death and ^{14}C radioactivity accumulated in excreta from time of feeding until death (disintegrations/min per estimated total disintegrations/min in all materials measured in that rat, $\times 10^2$) for two groups of six‡ individually-caged male rats per treatment killed 5·0 and 16·2 h respectively after access to food*

	Time (h) between access to food and death	Diet†			SEM	Statistical significance of difference:	
		JJ	JK	KK		JJ *v.* (JK+KK)/2	JK *v.* KK
Stomach	5·0	22	20	14	4·5	NS	NS
	16·2	0·41	0·52	0·33	0·054	NS	*
Small intestine							
1st segment	5·0	1·9	1·7	2·3	0·22	NS	NS
2nd segment	5·0	7·4	5·9	7·3	1·44	NS	NS
3rd segment	5·0	26·4	14·4	12·7	3·10	**	NS
Small intestine (total)	5·0	35·7	22·0	22·4	1·92	***	NS
	16·2	2·3	4·2	2·6	0·58	NS	NS
Caecum	16·2	63	64	69	3·3	NS	NS
Colon+rectum‖	5·0	0·5	3·6	2·0	1·01	NS	NS
	16·2	8·5	4·4	4·0	1·89	NS	NS
Large intestine (total)	5·0	24	32	39	3·6	•	NS
	16·2	72	68	73	2·3	NS	NS
Faeces‖	0–5·0	0·13	0·07	0·08	0·058	NS	NS
	0–16·2	0·1	0·1	2·1	0·34	*	**
Caecum–small intestine	5·0	0·75	1·32	1·78	0·185	**	NS
	16·2	28	17	28	3·3	NS	*
Urine	0–5·0	4·6	6·7	6·0	0·47	•	NS
	0–16·2	10·0	10·1	8·8	1·15	NS	NS
Blood plasma	5·0	7·6	10·3	10·4	0·56	**	NS
	16·2	6·5	7·9	6·5	0·55	NS	NS
Kidney	5·0	0·39	0·50	0·49	0·031	•	NS
	16·2	0·36	0·45	0·37	0·065	NS	NS
Liver	5·0	5·7	8·5	7·7	0·32	***	NS

NS, not significant ($P > 0·05$).
* $P < 0·05$, ** $P < 0·01$, *** $P < 0·001$.
† For details, see page 98.
‡ Three induced and three non-induced rats.
‖ Distribution of values highly non-normal, hence SEM and significance tests (included here for completeness) not reliable.

Total urinary ^{14}C in rats on diet JJ in the period 0–5·0 h was less than that in the other two ($P < 0·05$), whereas over 16·2 h no differences occurred. The pattern for the kidneys was similar. Thin layer chromatography and autoradiography failed to demonstrate differences between treatments in urinary metabolites, the principle one of which had an R_f value similar to aflatoxin M_1.

No clear treatment effect was observed for the total ^{14}C in the stomach, although there was some indication that the rats on diet KK retained less activity in this organ at 16·2 h. The activity passed out of the small intestine much more rapidly in the rats on diets JK and KK ($P < 0·001$). This was reflected in a greater ^{14}C ratio, caecum:small intestine for the rats on diets JK and KK ($P < 0·01$) at 5·0 h, as well as greater total ^{14}C activity in the large intestine ($P < 0·05$) and liver ($P < 0·001$) at this time.

Table 2. *Expt 1. Treatment mean total ^{14}C radioactivity (disintegrations/min per estimated total disintegrations/min in all materials measured in that rat, $\times 10^2$) at time of death of six individually-caged male rats per treatment*

		Diet				
		JJ	JK	KK	Mean	SEM†
Liver	Non-induced rats	6·0	10·8	7·0	7·9	1·47
16·2 h*	Induced rats	7·5	6·7	6·0	6·7	
	SEM‡		1·62			
	Mean	6·7	8·7	6·5		
	SEM‖		0·59			
Caecum	Non-induced rats	18	32	45	32	4·2
5·0 h*	Induced rats	30	24	28	27	
	SEM‡		5·5			
	Mean	24	28	37		
	SEM‖		3·0			

* Diet × induction interaction significant ($P < 0·05$).
† For comparing mean of induced and non-induced rats.
‡ For comparing mean of induced and non-induced rats on the same diet.
‖ For comparing diet means.

The small intestine with its contents was divided into three regions (proximal, middle and distal) and the total ^{14}C of the 5·0 h samples was assessed. No treatment differences occurred in the proximal or middle regions, but in the distal region the total ^{14}C content in rats on diet JJ was greater than that in the other two groups ($P < 0·01$).

There was an interaction between induced and non-induced rats and diet in the caecum ^{14}C at 5·0 h ($P < 0·05$) and in the liver ^{14}C at 16·2 h ($P < 0·05$). For rats on diet JJ, more ^{14}C occurred in these organs in the induced group at these times compared with the non-induced group, but the converse was found for the other two dietary treatments (Table 2).

Over all diets from 0–16 h there was no difference between induced and non-induced means, apart from those for caecal and urinary values. The mean ($\pm$SEM) percentage of total body counts accounted for in induced and non-induced rats was for the caecum 59 and $72 \pm 3·6$ respectively and for the urine 13·4 and $5·9 \pm 1·35$ respectively.

Expt 2

The rats were killed in replicates at approximately hourly intervals from 1·45 h after time of access to the ^{14}C-labelled aflatoxin B_1-treated meal until 6·6 h from time of access. A further replicate was killed 40·7 h after access. The latter group received two further non-radioactive meals before killing and was not used in the statistical analysis. The following results do not refer to this group except where explicitly stated as such.

Although differences existed amongst dietary treatments for carcass weight ($P < 0·001$) and liver weight ($P < 0·01$) (Table 3), covariance adjustment of liver weight by carcass weight removed the treatment effect on liver weight. There was a linear relationship ($P < 0·001$) between liver weight and time of killing which was the same for all treatments. Liver weight increased by approximately 0·58 g (11%) during the 5·1 h, and this effect was uninfluenced by differences in carcass weight.

A lower total hepatic ^{14}C activity was found in rats on treatment B than in either treatments A or H ($P < 0·05$). The mean difference between treatments A and B was

Table 3. *Expt 2. Treatment and time mean carcass and liver weights (g) and ^{14}C radioactivity (disintegrations/min, $\times 10^{-4}$) in entire organ (including contents of gastrointestinal tract), skeletal muscle mass and total urine excreted in six‡ individually-caged growing male rats per treatment each given $1{\cdot}482 \times 10^6$ disintegrations/min ^{14}C-labelled aflatoxin B_1 in their feed 1·45–40·7 h between access to food and death*

(Values in parentheses are adjusted by analysis of covariance to the over-all mean carcass weight)

	Wt		^{14}C					
	Carcass	Liver	Liver	Skeletal muscle	Urine‖	Stomach‖	Small intestine	Large‖ intestine
Diet‡								
A Starch + B_1†	128·5^{a}	5·22^{ab} (5·17)	11·8^{a}	34	3·9	43	44	14^{ab}
B Wheat offal + B_1†	119·7^{b}	5·06^{a} (5·26)	9·4^{b}	22	3·1	61	42	28^{a}
H Starch + chloroform–ethanol–water extract of offal + B_1†	131·7^{a}	5·54^{c} (5·40)	10·4^{a}	34	3·3	58	38	20^{ab}
L Starch + diethyl ether extract of offal + B_1†	127·5^{a}	5·35^{bc} (5·33)	10·3^{ab}	25	7·0	58	32	12^{b}
SEM	1·34***	0·075** (0·094)	0·49*	4·6	40%	13%	4·8	21%*
Time between access to food and death (h)								
1·45	128·3	5·09 (5·05)	7·6	31	0·1	125	31	2
2·4	123·3	4·95 (5·05)	9·0	35	4·9	101	46	6
3·3	128·3	5·18 (5·14)	9·9	34	6·3	96	47	15
4·3	129·0	5·33 (5·26)	11·5	19	13·0	46	49	26
5·4	127·3	5·52 (5·50)	11·8	30	15·4	27	29	67
6·6	125·0	5·67 (5·72)	12·8	22	18·4	18	33	78
40·7§	125·5	5·73 —	8·2	34	63·2	2	6	43
SEM	1·64	0·092*** (0·087***)	0·60***	5·7	51%***	16%***	5·9	27%***

a, b, c, Mean values in the same column that do not share a common superscript letter were significantly different ($P < 0{\cdot}05$). Over-all differences between diet means and between time means were significant: * $P < 0{\cdot}05$, ** $P < 0{\cdot}01$, *** $P < 0{\cdot}001$.

† Diet contained 1 mg aflatoxin B_1/kg during the last 10 d of the experiment.

‡ Excluding 40·7 h group.

‖ Analysis carried out on the logarithm of the actual value. The means on the original scale (given here) are therefore geometric rather than arithmetic and their standard error is expressed as a coefficient of variation.

§ Excluded from analysis of variance and calculation of standard errors.

Table 4. *Expt 3. Treatment mean carcass, liver and kidney weights (g/rat) of individually-caged male rats in two groups (1 and 2) with respectively seven and eight rats per treatment*

Group... Diet	Carcass wt		Liver wt		Kidney wt	
	1	2	1	2	1	2
A Starch	166	—	$7{\cdot}0^{ab}$	—	1·39	—
B Raw wheat offal	159	—	$6{\cdot}4^{a}$	—	1·34	—
C Starch + B_1†	165	185^{a}	$9{\cdot}2^{b}$	9·1	1·44	1·58
D Raw wheat offal + B_1†	162	165^{b}	$8{\cdot}2^{ab}$	8·3	1·44	1·52
E Autoclaved wheat offal + B_1†	163	176^{c}	$8{\cdot}3^{ab}$	7·9	1·36	1·50
G Starch + water extract of offal + B_1†	169	177^{c}	$8{\cdot}8^{ab}$	8·0	1·38	1·45
H Starch + chloroform–ethanol–water extract of offal + B_1†	174	190^{a}	$8{\cdot}3^{ab}$	8·3	1·30	1·40
SEM	3·2	2·3***	0·52*	0·56	0·050	0·049

a, b, c, Mean values in the same column that do not share a common superscript letter were significantly different ($P < 0{\cdot}05$).

Over-all differences between diet means were significant: $*P < 0{\cdot}05$, $***P < 0{\cdot}001$.

† Diet contained 2 mg aflatoxin B_1/kg for 16 weeks.

$2{\cdot}4 \times 10^4$ disintegrations/min. Liver activity increased uniformly with time up to 6·6 h ($P < 0{\cdot}001$) (Table 3) and the rate of increase was similar for all four diets. After the effects of killing time had been taken into account, there was no significant correlation within treatments between liver ^{14}C and liver weight. The ^{14}C activity in the total skeletal musculature was estimated from samples of thigh muscle. Amongst diet means this activity followed a similar pattern to that for liver, although the differences were not significant. However, in contrast to the effects on the liver no consistent trend with time was noted (Table 3). There was no significant correlation between muscle activity and carcass weight and an examination of it by thin-layer chromatography and autoradiography failed to reveal any ^{14}C radioactivity which was soluble in lipid solvents.

The total ^{14}C in the urine increased uniformly with time up to 6·6 h ($P < 0{\cdot}001$), but differences between treatment means were non-significant. Nevertheless, up to 5·4 h after access to the ^{14}C-treated meal a geometric mean of $2{\cdot}7 \times 10^4$ disintegrations/min (coefficient of variation 45%) accumulated in the urine of those receiving diet A, whereas only $2{\cdot}2 \times 10^4$ disintegrations/min (45%) were found for those on diet B.

Examination of the gastrointestinal tract revealed that the total ^{14}C in the stomach and its contents decreased uniformly with time ($P < 0{\cdot}001$) and at the same rate for each diet, but differences amongst the mean activities of the four diets were not significant (Table 3). No significant effect of diet could be detected in the ^{14}C activity of the small intestine and its contents, although a quadratic trend with time was apparent in the data with a maximum at 4·3 h ($P < 0{\cdot}05$). The ^{14}C in the large intestine increased with time ($P < 0{\cdot}001$). In rats killed between 1·45 and 5·4 h a geometric mean of only $1{\cdot}0 \times 10^5$ disintegrations/min (23%) were recovered from the large intestines of those on diet A whereas $2{\cdot}2 \times 10^5$ disintegrations/min (23%) were found in those on diet B. At 6·6 h the values for A and B were $7{\cdot}4 \times 10^5$ (60%) and $9{\cdot}2 \times 10^5$ (60%) respectively, and at 40·7 h the values were $13{\cdot}2 \times 10^5$ (60%) for diet A and only $0{\cdot}9 \times 10^5$ (60%) for diet B. Differences amongst diet means up to 6·6 h for the large intestine were significant ($P < 0{\cdot}05$): diet B produced higher activities than diet L. Covariance adjustment by carcass weight was not significant for any region of the gastrointestinal tract.

Table 5. *Expt 3. Treatment mean total ^{14}C radioactivity (disintegrations/min, $\times 10^{-4}$) in urine, faeces and liver of seven‡ individually-caged male rats per treatment following the consumption of a ^{14}C-labelled aflatoxin meal*

	Total urine				Total faeces				Whole liver
Time after dosing (h)... Diet	0–19	19–48	48–168	0–168	0–48	48–96	96–168	0–168	at death
C Starch + B_1†	24·0	6·5[a]	5·5[a]	36·0[a]	83[a]	19	3·7[a]	105[a]	9·9[a]
D Raw wheat offal + B_1†	21·1	3·1[b]	3·8[b]	27·9[b]	102[b]	13	2·8[b]	117[b]	8·3[b]
H Starch + chloroform–ethanol–water extract of offal + B_1†	22·0	5·5[a]	5·4[a]	32·9[c]	77[a]	19	3·9[a]	100[a]	9·5[a]
SEM‖	0·79	0·48***	0·18***	0·71**	3·0***	5·0	0·22*	3·5*	0·31*
A Starch	18·7	6·7	7·5	32·8	46	26	6·5	78	8·7
B Raw wheat offal	19·5	4·2	5·9	29·6	88	7	5·1	101	7·5

a, b, c, Mean values in the same column that do not share a common superscript letter were significantly different ($P < 0·05$).

Over-all differences between means of diets C, D and H were significant: *$P < 0·05$, **$P < 0·01$, ***$P < 0·001$.

† Diet contained 2 mg aflatoxin B_1/kg for 16 weeks.

‡ Diets C, D and H; one rat on each of diets A and B.

‖ Diets C, D and H; SEM for diets A and B can be taken as 2·65 times that given for diets C, D and H.

Expt 3

Rats which had received the starch diet plus aflatoxin (diet C) continuously for 114 d produced heavier livers ($P < 0·05$) than did those which had received that containing raw wheat offal (diet B) (Table 4). However, amongst the rats of group 2 receiving the diets containing aflatoxin, rats which had received the starch diet (diet C), or that containing starch plus the lipid extract of wheat offal (diet H), had heavier carcasses than those on the other diets. No effect of treatment on kidney weight was observed.

During the first 48 h after dosing with ^{14}C-labelled aflatoxin very much more activity appeared in the faeces of rats which had received raw wheat offal (diets D and B) compared with the appropriate controls (diets C and A) regardless of whether they had or had not previously received non-radioactive aflatoxin (Table 5). The faecal ^{14}C losses in these rats were less in the period 96–168 h. Over-all, more ^{14}C activity was found in the faeces of rats receiving raw wheat offal ($P < 0·05$). The chloroform–ethanol–water extract had no effect on this characteristic (diet H *v.* diet C). The majority of the urinary losses of ^{14}C occurred during the first 19 h after dosing, but the urinary losses of rats which had received raw wheat offal were less than those of rats on other diets in the periods 19–168 h ($P < 0·001$) and over-all 0–168 h ($P < 0·01$) (Table 5). The ^{14}C activity per liver was least amongst rats which had received raw wheat offal whether they had been given non-radioactive aflatoxin or not ($P < 0·05$), the extract of offal (diet H) having no effect.

Plasma lipids were elevated in rats which had received aflatoxin or starch. In the presence of aflatoxin, raw wheat offal, but not its water extract, tended to redress the effect (P for significance of over-all treatment effects = 0·056) (Table 6).

Expt 4

Rats fed on day of death. The rats were killed in replicates 5·0, 5·3 and 5·7 h after the meal containing the radio-tracer was given. Although diet means for liver weight and carcass weight were related, there was no significant correlation within diets and so no adjustment of liver weight for carcass weight was made. Differences occurred amongst treatments in

Table 6. *Expt 3. Treatment mean blood plasma lipid values (mg/l) at death in individually-caged male rats in two groups (1 and 2) with respectively five and six rats per treatment*

	Triglycerides		Total cholesterol	
Group... Diet	1	2	1	2
A Starch	1872	—	683[a]	—
B Raw wheat offal	1590	—	679[a]	—
C Starch + B_1†	2578	1626	765[ab]	743[a]
D Raw wheat offal + B_1†	1889	1405	729[ab]	648[b]
E Autoclaved wheat offal + B_1†	2032	1978	816[ab]	766[a]
G Starch + water extract of offal + B_1†	1917	1597	861[b]	835[c]
H Starch + chloroform–ethanol–water extract of offal + B_1†	2122	1790	738[ab]	704[ab]
SEM	185	155	31**	22***

a, b, c, Mean values in the same column that do not share a common superscript letter were significantly different ($P < 0.05$).

Over-all differences between diet means were significant: **$P < 0.01$, ***$P < 0.001$.

† Diet contained 2 mg aflatoxin B_1/kg for 16 weeks.

Table 7. *Expt 4. Treatment mean carcass and liver weights (g) and ^{14}C radioactivity (disintegrations/min, $\times 10^{-4}$) for liver, stomach, large intestine including contents and estimated skeletal muscle mass of six individually-caged male rats per treatment each given $1{\cdot}725 \times 10^6$ disintegrations/min ^{14}C-labelled aflatoxin B_1 in 3 g diet 5·0–5·7 h previously*

			^{14}C				
Diet	Carcass weight	Liver weight	Liver	Skeletal muscle‡	Stomach	Large intestine	Urine
B Starch + B_1†	109·5[a]	5·46[a]	21·9[a]	5·6	40	112	20·7
C Wheat offal + B_1†	99·0[b]	4·98[b]	16·6[b]	4·3	65	130	19·5
D Starch + fibre + B_1†	97·0[b]	5·05[b]	15·1[b]	4·3	76	112	16·2
E Starch + oil + B_1†	109·7[a]	5·43[a]	22·7[a]	4·5	33	94	20·5
SEM	1·04***	0·085**	0·96***	0·34	12·5	14·6	1·72

a, b, Mean values in the same column that do not share a common superscript letter were significantly different ($P < 0.05$).

Over-all differences between diet means were significant: **$P < 0.01$, ***$P < 0.001$.

† Diet contained 0·5 mg aflatoxin B_1/kg for 34 d.

‡ Skeletal muscle mass assumed to be 0·5 times carcass weight.

carcass weight ($P < 0.001$) and in liver weight ($P < 0.01$) (Table 7). The rats receiving the wheat offal (diet C), or MAD fibre extract (diet D) possessed carcasses 10% lighter and livers 8% lighter than were found in the other two treatment groups. Liver ^{14}C was 29% less for treatments C and D than for the other two and, after replicate effects had been taken into account, there was no significant relationship within diets between liver ^{14}C and liver weight (Table 7). Treatment differences in liver and carcass weight are likely to have been functions of differences between diets in metabolizable energy; but treatment differences in liver ^{14}C-aflatoxin are not likely to have been related to dietary energy, nor was the retention of a single tracer dose likely to have been a physiological function of liver size in these circumstances. Thus the lack of within diet correlation referred to above is not unexpected.

Table 8. *Expt 4. Treatment mean blood plasma lipid values (mg/l) in individually-caged male rats fasted (four‡ rats per treatment) and those not fasted (six rats per treatment) on day of death (data for each group separately)*

Diet	Triglycerides		Total cholesterol	
	Fasted	Fed	Fasted	Fed
A Starch	546	—	432[ab]	—
B Starch + B_1†	547	1804	405[a]	745
C Wheat offal + B_1†	483	1622	439[ab]	840
D Starch + fibre + B_1†	408	1446	468[b]	801
E Starch + oil + B_1†	465	1920	473[b]	848
SEM	56‖	118	12·4**§	29·1

a, b, Mean values in the same column that do not share a common superscript letter were significantly different ($P < 0.05$).

Over-all differences between diet means were significant: $^{**}P < 0.01$.

† Diet contained 0·5 mg aflatoxin B_1/kg for 34 d.

‡ Diets B–E; eight rats on diet A.

‖ Diets B–E; SEM for diet A = 40.

§ Diets B–E; SEM for diet A = 8·7.

Total ^{14}C in the stomach and large intestine was quite variable, and treatment differences non-significant, but rats receiving a fibre source (diets C and D) tended to have more ^{14}C in those organs than did rats on the other treatments. Urine ^{14}C values tended to parallel the liver values but the variability was too great for this relationship to attain a level of significance. There was a tendency for less ^{14}C to be present in the total skeletal musculature of rats on treatments C and D (wheat offal and fibre) than in those on B (starch), but the differences were not significant. There was no significant correlation within treatments between muscle ^{14}C and carcass weight, although there was a positive correlation with liver ^{14}C ($P < 0.05$). Total liver plus muscle ^{14}C for rats on diets C and D was found to be only 74% of that accumulated by the other two diets.

Analysis of the time effects showed that muscle activity was declining during the interval between 5·0 and 5·7 h after the feed was offered ($P < 0.01$). In this experiment half the replicates were given access to their feed at 08.05 hours and half at 09.05 hours with the interval between then and death remaining the same. However, those fed at 09.05 hours on average had 37% less muscle ^{14}C than did those fed at 08.05 hours ($P < 0.001$).

No significant treatment effects on blood plasma lipids were observed amongst rats which had been fed on the day of death (Table 8).

Rats fasted for 24–30 h before death. No significant differences in plasma triglycerides occurred amongst treatments, although the mean values conformed with those found in Expt 3. Blood plasma total cholesterol was lower in rats receiving diet B than in those receiving diets D or E. Rats receiving the basal starch diet containing no aflatoxin (diet A) held an intermediate position.

The results of the mutagenesis assay, using two tester strains of *S. typhimurium* confirmed that the addition of the S-9 fraction to the medium was required in order to obtain extensive mutagenesis (162 *v.* 59 revertant colonies per plate): the mutagenic frequency being directly related to the S-9 concentration at non-toxic levels of aflatoxin in agreement with Suit *et al.* (1977). A low incidence of mutagenesis was apparent in the absence of aflatoxin but mutagenic activity was related to aflatoxin concentration of the medium at non-toxic aflatoxin concentrations.

Table 9. *Expt 4. Square root of revertant colony counts for aflatoxin concentrations (μg/plate) and dietary source of S-9 fraction as the mean of tester strains T98 and TA100*

Diet	Aflatoxin concentration						
	0·50	0·20	0·10	0·05	0·02	0·01	0
A Starch	31	26	23	16	13	12	8
B Starch + B_1†	30	27	20	14	12	12	8
C Wheat offal + B_1†	31	33	21	17	13	12	8
D Starch + fibre + B_1†	28	27	21	15	12	14	8
E Starch + oil + B_1†	35	29	22	19	14	12	8

SEM for comparing different aflatoxin concentrations within the same diet ±0·98. (No SEMs are available for comparisons involving different diets).

† Diet contained 0·5 mg aflatoxin B_1/kg for 43 d.

The inclusion of aflatoxin B_1 in the diet had no effect on the efficacy of the S-9 fractions (diet A *v.* diet B) (Table 9). Ingredient composition of the diets B, C and D had no apparent effect on the mutagenic activity which could be correlated with the distribution of ^{14}C.

DISCUSSION

Several alternative mechanisms could explain the observations made. First, many investigations have hinted that the enterohepatic cycle of bile acids is affected by certain sources of dietary fibre. A depression of serum and hepatic cholesterol (Chen & Anderson, 1979; Stasse-Wolthius, 1979) by dietary fibre is probably a consequence of a stimulation to the synthesis (Brydon *et al.* 1980) and secretion of bile acids (Burkitt & Trowell, 1975). In vitro it has been demonstrated that dietary fibre can bind bile salts, and such a process occurring in the intestinal lumen would interfere with their enterohepatic circulation. This in turn would stimulate hepatic synthesis of the acids from cholesterol, causing hypocholesterolaemia (Kritchevsky *et al.* 1975). However, cellulose and wheat bran have been shown in vitro to be much less effective in binding bile salts and in reducing serum cholesterol than are pectin, lignin, lucerne, and guar gum (Kritchevsky & Story, 1974; Story & Kritchevsky, 1976; Kay & Truswell, 1977; Chang & Johnson, 1978; Johnson & Chang, 1978; Lin & Anderson, 1978); and wheat bran has no effect on microsomal cholesterol 7αhydroxylase (*EC* 1.14.13.17) activity (Brydon *et al.* 1980). Our experiments also failed to elicit any hypocholesterolaemic effect from wheat offal, but this by-product had no less, and probably has a greater, ameliorating effect on the toxicity of aflatoxin than does pectin, cellulose, gum arabic and lucerne (Frape *et al.* 1981). Evidence (Truswell & Kay, 1976; Oakenfull & Fenwick, 1978; Oakenfull *et al.* 1979) indicates that only fibre preparations containing saponins (not contained in wheat bran) increase the rate of bile acid synthesis and lower plasma cholesterol. Other evidence (Ranhotra *et al.* 1978) has shown that rats given a lipogenic diet develop lower concentrations of serum cholesterol and liver triglycerides when they also receive full-fat wheat bran in place of sucrose, whereas defatted bran failed to prevent an elevation in the concentration of these determinants. In our experiment the lipid extracts of wheat bran had no effect on aflatoxin metabolism, but the MAD fibre extract was at least as effective as the full-fat offal in influencing its metabolism without decreasing serum cholesterol concentration. Thus the mechanism of this effect appears to be independent of an influence of certain fibre sources on lipid metabolism.

Patterson (1973) proposed that the hydroxy-derivatives of aflatoxin B_1: M_1, P_1 and Q_1 are partially detoxified by conjugation with taurocholic and glucuronic acids with subsequent

excretion in bile or urine. However, any effect of dietary change on this route of elimination is unlikely to have been a part of the mechanism of wheat fibre action observed in these experiments. Hepatic and urinary accumulation of ^{14}C was less and faecal elimination was increased by wheat offal. Despite a direct relationship between in vitro S-9 concentration and mutagenic rate, neither dietary fibre, nor attempted induction of the rats with dietary aflatoxin B_1 had any effect on the production of mutagenic products by hepatic microsomal enzymes in Expt 4. On the other hand, diet has been shown to influence the hepatic metabolic pathways of aflatoxin (Newberne & Rogers, 1976; Suit *et al.* 1977). It is possible that chronic effects of aflatoxicosis in rats are mediated by microsomally-produced metabolites other than those which induce mutation in vitro. Alternatively extramicrosomal enzymes affected by diet may be involved (a possibility strengthened by the effect of induction on ^{14}C distribution in Expt 1, where the results suggest the possibility that induced rats more effectively disposed of aflatoxin from the liver). Thus these experiments do not exclude the possibility that dietary fibre can indirectly mediate in the hepatic metabolism of aflatoxin, but they make that possibility less likely.

The values presented here and those given by Frape *et al.* (1981) clearly show that compared with starch both gum arabic and wheat offal accelerated the rate of passage of ^{14}C through the gastrointestinal tract, both to the large intestine and into the faeces. Despite similarities between gum arabic and wheat offal in these respects, only wheat offal lowered the hepatic retention of ^{14}C. The rate of increase of ^{14}C in the urine was accelerated by gum arabic but not by wheat offal; rate of passage and of absorption both affecting urinary elimination of many dietary entities. This observation compares with that of Brown *et al.* (1979) that pectin but not wheat bran increased the rate of absorption and urinary excretion of Paracetamol.

In the pig 80% of the total mean transit time is represented by retention in the large intestine (Keys & De Bartha, 1974) and it is assumed therefore that the major effect of fibre is to increase mobility of, and reduce retention time in, the hind gut (Fioramonti & Bueno, 1980). Our results (Expts 1 and 2) show that rate of passage of ^{14}C through the small intestine of the rat is also accelerated by dietary fibre, although retention time in the large intestine is greatly diminished, in particular by wheat offal (Expts 1, 2 and 3 in the present study and Expt 4 reported by Frape *et al.* 1981). However, this did not affect the rate at which hepatic ^{14}C accumulated in rats on diet B compared to those on diet A. The ratio, mean diet A:mean diet B, for total hepatic ^{14}C in Expt 2 was 1·21 between 1·1 and 2·9 h after consuming the meal and 1·28 between 3·9 and 6·2 h after consumption. Thus less hepatic ^{14}C was found in offal-fed rats even before the rate of removal of metabolites from the liver exceeded the rate of accumulation. Hepatic concentration would be influenced by the rate at which digesta reach absorption sites, the efficiency of that absorption and the rate at which metabolites capable of urinary and biliary excretion are produced. Less ^{14}C accumulated during the first 5 h and, in total, in the urine of rats receiving wheat offal than in those receiving starch. It is therefore concluded that wheat offal depressed the efficiency of ^{14}C absorption from the intestines. This may have resulted from an interruption of the absorptive process, or from the digesta passing the sites of absorption more rapidly. Our results obtained from the consumption of gum arabic showed that an accelerated rate of passage itself had no effect on liver ^{14}C accumulation. Faecal DM was also less in rats given gum arabic than in those given wheat offal and it is concluded that this offal had a specific effect on the efficiency of absorption of aflatoxin from the intestine. The deficit of ^{14}C in the livers, skeletal muscles and urine of offal-fed rats in Expt 2 was accounted for to a large extent by the higher activity in the gastrointestinal tract (diet B *v.* diet A).

The MAD-fibre extract of wheat offal appeared to account for the effect of the offal entirely. This extract contains very little hemicellulose (Southgate, 1976) and hence the active

component appears to reside in the remainder of the fibre fraction, possibly accounting for the nil effect of gum arabic, a major source of hemicelluloses.

The results of the present experiments show that certain constituents of diet which may be considered inert, or not contributing directly to its nutritive value, can indirectly affect that value. Such constituents could in part account for variation in outcome between drug toxicity tests despite a dietary similarity in nutrient concentration, but a dissimilarity in ingredient make-up.

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