Dietary Factors and Aflatoxin Toxicity: I. Comparison of the Effect of Two Diets Supplemented with Aflatoxin B_1 upon Two Different Strains of Rats

R.B. ALFIN-SLATER, L. AFTERGOOD, and P. WELLS, School of Public Health, University of California, Los Angelas, California 90024, D. MELNICK, Best Foods Research Center, CPC International Inc., Union, New Jersey 07083, and R. STRAUS, Providence Hospital, Portland, Oregon 97213

ABSTRACT

Previous studies in this laboratory with rats fed low levels of aflatoxin as a component of peanut discards, suggested either a strain tolerance for aflatoxin or a possible protective factor present in the diets used. In this study, two strains of rats, the Charles-River strain and the former USC strain, were used to test the effect of 1.7 ppm purified aflatoxin B_1 included for 3 months in two different diets; one previously used in this laboratory and one used by other investigators in aflatoxin studies. After an experimental period of either 12 or 18 months, growth, mortality, gross pathology, and organ wt were measured, and histopathological examination and biochemical analyses were performed. Plasma and liver cholesterol levels, total liver lipids, and fatty acids in the various lipid fractions of plasma, liver, and liver tumor lipids were measured. Both strains of rats proved to be susceptible to aflatoxin toxicity at this level as manifested by the appearance of hepatomas; however, liver involvement was more extensive in the Charles-River rats. The diet used by other investigators produced symptoms similar to those observed as a result of essential fatty acid deficiency and also affected the response to aflatoxin through an aggravation of symptoms, i.e. an inhibition of growth and increased size and severity of the liver tumors.

TABLE I

Plan of the Experiment

Group[a]				
No.	Months	Strain of Rat	Aflatoxin B_1[b] (ppm)	Diet
CA-II	12,18	Charles River	1.7	I
CC-I	12,18	Charles River	0	I
CA-II	12,18	Charles River	1.7	II
CC-II	12,18	Charles River	0	II
AA-I	12,18	Alfin-Slater (USC)	1.7	I
AC-I	12,18	Alfin-Slater (USC)	0	I
AA-II	12,18	Alfin-Slater (USC)	1.7	II
AC-II1	12,18	Alfin-Slater (USC)	0	II

[a]Each group contained 12 male weanling rats.

[b]Fed for first 3 months.

TABLE II

Comparison of the Two Basal Diets Used

Diet I	Percent	Diet II	Percent
Peanut butter	35.00	Casein	7.00
Ground whole wheat	43.50	Peanut meal[c]	34.00
Lactalbumin	5.08	Sucrose	32.00
Skim milk powder	15.00	Salt mix[d]	5.00
Salt	1.00	Vitamin mix[e]	2.00
Calcium carbonate	0.50	Lard	20.00
α-Tocopherol[a]	0.04		
Crystalets[b]	0.0015		

[a]1 mg = 0.25 IU as α-tocopheryl acetate.

[b]500 IU vitamin A and 50 IU vitamin D/mg.

[c]The peanut butter of diet I (commercial product obtained from CPC International, Union, New Jersey) after removal of the oil phase (see text).

[d]Wesson modification of the Osborne and Mendel salt mix (14).

[e]Ref. 15.

INTRODUCTION

Since aflatoxin was first isolated from peanut meal during an investigation of the epizootic of "Turkey X" disease in England in 1961, the acute and chronic effects of the toxin and its metabolites have been studied in detail. Aflatoxins are toxic to a wide range of animal species and in at least five (rat, ferret, duck, trout, and infant mouse), carcinogenicity has been demonstrated (1-4). This naturally gives rise to obvious and important questions concerning the risks to human health posed by these and other foodborne, mold-produced toxins, particularly since many human populations seem to be regularly, albeit inadvertently, consuming aflatoxin contaminated foodstuffs (5,6).

It has been reported by numerous investigators that the toxic effects of aflatoxin may be either alleviated or potentiated by dietary manipulation. The presence of cyclopropenoid fatty acids, which are natural minor components of cottonseed, in the diets of rainbow trout has been reported to enhance the carcinogenic effect of minute amounts of aflatoxin (7). The addition of certain amino acids to diets fed to ducklings has been reported to decrease mortality resulting from aflatoxin administration (8). Diets low in lipotropic factors have been reported to potentiate the toxic effects of aflatoxin on the liver of rats (9) as have diets which are cirrhotic in nature (10). Varying the protein content of the diet also appears to affect susceptibility to aflatoxin toxicity, although the reports are contradictory (11,12).

Previous studies in this laboratory with our strain of rats (former USC strain), fed aflatoxin as peanut discards at the 10 and 80 ppb level, have shown an apparent resistance to tumor formation (13). These rats not only grew as well, but, in fact, those at the lower level of aflatoxin intake had a higher rate of survival than did the control animals. These studies suggested either a strain tolerance for aflatoxin or, possibly, the presence of a protective factor in the diets fed and, therefore have led to the present studies in which two different strains of rats and two different diets were used.

EXPERIMENTAL PROCEDURES

The plan of the experiment is shown in Table I. Male weanling rats of the former USC strain, referred to here as Alfin-Slater rats, and of the Charles River strain were fed aflatoxin B_1 at a level of 1.7 ppm for 3 months and thereafter were fed a control diet without aflatoxin until sacrifice at either 12 or 18 months of age. Each group contained 12 animals.

The diets are shown in Table II. The animals were fed

either the 35% peanut butter diet previously used in our laboratory (diet I); or a diet (diet II), used by others in aflatoxin feeding studies with rats in which hepatomas were reported with rather low levels of aflatoxin contamination (15).

The peanut meal of diet II was prepared from the peanut butter of diet I. This involved repeated extractions of the peanut butter with hexane at 38 C and elimination of the solvent from the filter cake by forced draft air ventilation but with no heat applied. The meal contained less than 25 ppm of residual hexane and fat had been reduced from 50 to 0.5%.

Diet II differs from diet I primarily in its limited protein source, and the different fat source. The varied natural protein sources in diet I may provide a more satisfactory amino acid mixture and other nutrients for the animal than does the protein used in diet II. The fat source in diet I provides 5% linoleic acid as compared to 2% in diet II. In addition, the synthetic vitamin mixture used in diet II, while adequate for growth in rats, does not contain either p-aminobenzoic acid or biotin.

During the experimental period, records were kept of growth, morbidity, and mortality. At the time of sacrifice, gross, as well as histopathological, examinations of tissues were conducted, and organ wt were compared. Cholesterol determinations, thin layer separation of lipid fractions and gas liquid chromatographic analysis were performed on various tissues as described previously (16,17).

RESULTS AND DISCUSSION

It is immediately apparent, from observations on growth and wt gain (Fig. 1), that a strain difference exists between the Charles River and Alfin-Slater rats. With both strains given the same diet ad libitum, the Charles River rats were remarkable for their tendency to become extremely obese. Not only was the growth of Charles River rats more pronounced in its response to the type of diet fed, but it was also more variable. Regardless of species or diet, however, it can be seen that the presence of aflatoxin at this level in the diet (1.7 ppm) markedly inhibits growth.

At autopsy, varying degrees of liver pathology were found in all rats fed aflatoxin-containing diets (Table III). This pathology consisted primarily of greyish fatty nodules of varying size, cystic areas filled with straw-colored fluid, and generalized fatty degeneration of the liver tissue.

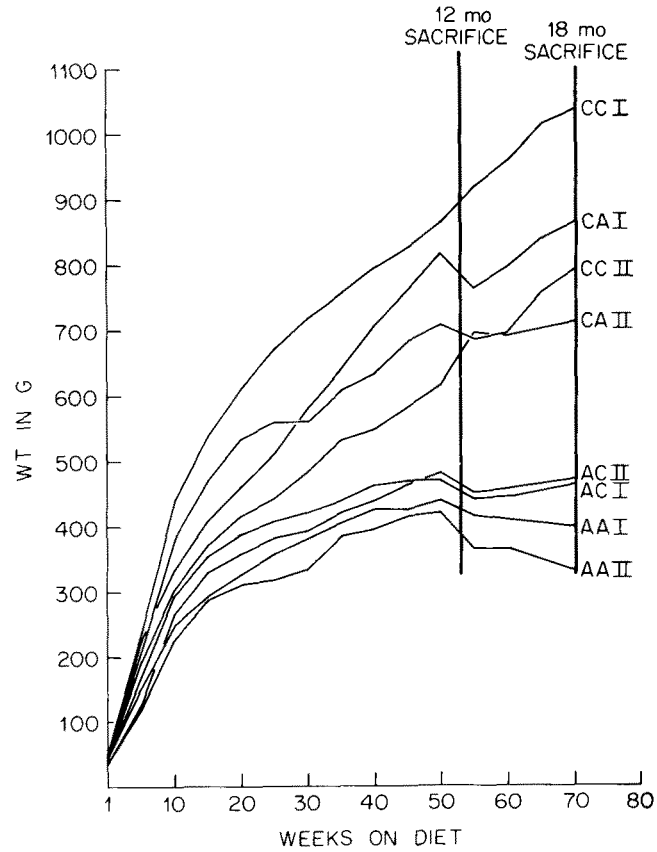

FIG. 1. Summary of growth of Charles River and Alfin-Slater rats fed two different diets with and without aflatoxin B_1.

Among Charles River rats, fatty degeneration of the liver was present in the control, as well as in the experimental group, due probably to the obesity of the animals. Nontumorous areas of liver tissue in aflatoxin-fed Charles River rats were mottled in color and irregular in texture, whereas the areas of nontumorous liver tissue in the aflatoxin-fed Alfin-Slater rats, more closely resembled the livers of the controls. It is noteworthy that although liver involvement among the aflatoxin-fed animals, regardless of diet or strain, often had progressed to the point where there was no normal tissue at all, tumor masses of the type

TABLE III

Summary of Gross Pathology of Charles River and Alfin-Slater Rats Fed Two Different Diets with and without Aflatoxin

Group[a]	g	% of body wt	Liver: Fatty degeneration	Liver: Cysts and fatty nodules	Liver: Complex tumor mass
CA I-12 (6)	29.0	3.2	5 (83%)	3 (50%)	---
CA I-18 (4)	43.0	4.8	4 (100%)	3 (75%)	1 (25%)
CC I-12 (6)	21.3	2.5	2 (33%)	---	---
CC I-18 (3)	25.0	2.2	3 (100%)	---	---
CA II-12 (7)	23.2	3.8	5 (71%)	6 (85%)	---
CA II-18 (4)	47.8	6.6	3 (75%)	4 (100%)	1 (25%)
CC II-12 (6)	19.8	2.5	---	---	---
CC II-18 (3)	20.9	2.5	2 (66%)	---	---
AA I-12 (5)	17.4[b]	4.0	2 (40%)	5 (100%)	2 (40%)
AA I-18 (5)	20.8[c,f]	5.0	5 (100%)	5 (100%)	2 (40%)
AC I-12 (6)	11.1[b]	2.3	---	---	---
AC I-18 (6)	11.1[c]	2.3	---	---	---
AA II-12 (6)	21.9[d]	5.2	1 (16%)	6 (100%)	2 (33%)
AA II-18 (4)	56.6[e,f]	15.6	4 (100%)	4 (100%)	3 (75%)
AC II-12 (6)	11.6[d]	2.3	---	---	---
AC II-18 (6)	11.7[e]	2.4	---	1 (16%)	---

[a]Numbers in parenthesis are number of animals/group.

[b-f]Matched superscripts indicate significant differences at $p < .025$.

TABLE IV

Plasma Cholesterol and Liver Lipids of Charles River and Alfin-Slater Rats Fed Two Different Diets with and without Aflatoxin

Group	Plasma cholesterol	Liver		
	Total, mg %	Total lipid mg/g	Total cholesterol mg/g	% free
CA I-12	114.6 ± 41.0	78.0 ± 34.3	6.02 ± 2.60[g]	32
CC I-12	102.1 ± 15.4	53.2 ± 12.3	2.53 ± 0.19[g]	62
CA II-12	78.2 ± 19.9	82.9 ± 23.9[e]	5.47 ± 1.32[h]	26
CC II-12	88.7 ± 21.1	43.9 ± 14.7[e]	2.07 ± 0.70[h]	68
AA I-12	87.6 ± 8.3[a]	51.0 ± 5.5	3.27 ± 0.66	74
AA I-18	114.2 ± 11.0[b]	42.3 ± 8.5[f]	2.42 ± 0.64	75
AC I-12	73.4 ± 8.3[a]	46.0 ± 13.2	2.90 ± 0.30	66
AC I-18	82.1 ± 3.3[b]	55.5 ± 5.8[f]	2.43 ± 0.31	88
AA II-12	103.9 ± 9.5[c]	52.6 ± 5.7	4.42 ± 0.60	47
AA II-18	139.2 ± 18.5[d]	45.0 ± 10.9	3.74 ± 1.30	68
AC II-12	72.4 ± 9.2[c]	57.0 ± 5.9	4.64 ± 0.70	50
AC II-18	85.3 ± 5.7[d]	55.4 ± 12.4	2.84 ± 0.91	70

[a-h]Matched superscripts indicate significant differences at $p < .025$.

TABLE V

Major Fatty Acids of the Sterol Esters Fraction of Pooled Plasma of Charles River and Alfin-Slater Rats Fed Two Different Diets with and without Aflatoxin

Group	Fatty acids (%)					
	18:0	18:1	18:2	20:3	20:4	20:3/20:4
CA I-18	1.7	9.5	14.8	Trace	54.9	---
CC I-18	5.9	14.6	20.4	---	38.3	---
CA II-18	2.8	29.4	10.4	6.4	25.5	.25
CC II-18	2.8	14.0	17.8	7.4	42.5	.17
AA I-18	1.9	17.9	22.6	---	37.3	---
AC I-18	6.8	20.9	22.0	---	22.2	---
AA II-18	1.4	40.5	12.8	6.8	12.2	.56
AC II-18	1.3	29.3	11.8	8.4	22.3	.38

previously described by other investigators as being carcinoma, namely, "soft, friable nodules with hemorrhagic and necrotic areas; structures which were moderately to poorly formed with little if any differentiation and direct extension of the hepatomas to the mesentery and metastases to the lungs" (18), were observed only infrequently in our investigation. In several cases, however, it appeared that metastases to other organs had occurred.

Gross examination revealed that among the Alfin-Slater rats, there was more severe liver involvement in animals fed diet II supplemented with aflatoxin than in those fed diet I supplemented with aflatoxin. This was reflected by increased size of the tumors, as well as a greater number of nodules/liver. Increased mortality and some kidney lesions were observed in the Charles River rats, but this appeared to be a function of their obesity rather than of the toxic components of diet.

Histological findings indicated that, among animals sacrificed at 12 months, abnormal changes were minimal in Charles River rats fed the aflatoxin-containing diets and were apparent primarily among those animals fed diet II. These changes consisted primarily of moderate focal adiposity, distended sinusoid cells which varied in size with the zones of change being poorly circumscribed. Aflatoxin-fed Alfin-Slater rats on both diets, on the other hand, all demonstrated some liver involvement and, in several cases, tumors also were observed in kidney and adrenals. Examinations of spleen, stomach, heart, aorta, and testes all proved negative.

At the time of the 18 month sacrifice, differences in response to aflatoxin as a result of diet were readily apparent histologically. All Charles River rats fed diet II with aflatoxin exhibited multiple hepatomas, with possible metastases to the kidney in several cases. Tumor nodules in liver were composed of both parenchymal cells and duct-like structures. The cells were large, pleomorphic, and some were hyperchromic. The tumors in kidney were also pleomorphic, but composed chiefly of the hepatic parenchymal cells with little evidence of ducts. Only one case with metastasis was observed among Charles River animals fed diet I. In Alfin-Slater rats, these marked differences in liver pathology as a result of diet, were not observed. All of the rats on both diets appeared to be affected equally by aflatoxin administration.

Liver wt reflected the presence of tumors. The wt of other organs showed no significant differences attributable to either the diet or the presence of aflatoxin. In all cases, aflatoxin-fed rats had larger livers, absolutely and relative to body wt, than did the controls; this enlargement became more pronounced with age. The differences, however, were statistically significant only among Alfin-Slater rats and were most pronounced among rats fed diet II.

Total liver lipids and plasma and liver cholesterol values for the rats sacrificed at 18 months are shown in Table IV. In the early stages of this study, liver lipid analyses were conducted on the total liver remaining after slices had been removed for fatty acid analysis and histology, as is customary in our laboratory. At the time of the 18 month sacrifice of the Charles River rats, however, the livers were so greatly affected that this procedure resulted in highly variable data making statistical evaluation difficult. These data have not been included since the large standard deviations made meaningful comparisons impossible. It subsequently was decided that data on uninvolved liver tissue would be of more value in defining biochemical changes which might occur in liver prior to and during aflatoxicosis. Consequently, when the Alfin-Slater rats were sacrificed at 18 months, uninvolved liver tissue was separated and analyzed separately from areas of cystic infiltration and tumor masses.

TABLE VI

Major Phospholipid Fatty Acids of Pooled Plasma of Charles River and of Alfin-Slater Rats Fed Two Different Diets with and without Aflatoxin

Group	Fatty acids (%)						
	16:0	16:1	18:0	18:1	18:2	20:3	20:4
CA I-18	14.0	1.6	12.9	19.4	15.7	4.8	8.0
CC I-18	13.1	0.9	27.2	12.4	9.6	2.3	24.1
CA II-18	17.9	0.8	14.8	21.2	14.1	5.5	4.9
CC II-18	17.9	5.2	12.5	12.7	8.8	5.8	15.3
AA I-18	23.0	8.4	6.0	24.1	8.0	---	4.8
AC I-18	30.6	7.7	12.3	29.9	8.8	---	Trace
AA II-18	29.1	6.7	3.6	16.5	7.2	---	Trace
AA II-18	26.0	13.5	8.3	23.8	3.3	---	6.0

TABLE VII

Major Fatty Acids of the Liver Sterol Esters Fraction of Charles River and Alfin-Slater Rats Fed Two Different Diets with and without Aflatoxin

Group	Fatty acids (%)				
	16:0	18:0	18:1	18:2	20:4
CA I-18	13.2 ± 4.1	4.7 ± 1.2[c]	49.6 ± 6.8	12.8 ± 0.7	8.4 ± 1.5[e]
CC I-18	11.2 ± 2.1	6.8 ± 0.8[c]	35.8 ± 9.5	12.0 ± 2.6	12.8 ± 2.1[e]
CA II-18	14.8 ± 1.4	5.8 ± 2.2	50.9 ± 12.9	4.6 ± 2.8	3.9 ± 3.5
CC II-18	22.8 ± 7.0	5.1 ± 1.7	43.3 ± 6.6	5.8 ± 0.8	5.9 ± 1.6
AA I-18	11.6 ± 2.7[a]	5.6 ± 1.3	39.4 ± 6.6[d]	11.4 ± 1.6	6.9 ± 1.6
AC I-18	20.1 ± 1.3[a]	8.0 ± 3.8	29.3 ± 1.3[d]	10.0 ± 0.6	9.1 ± 2.1
AA II-18	21.1 ± 2.9[b]	6.6 ± 1.5	38.2 ± 1.3	6.0 ± 1.6	2.6 ± 2.0
AC II-18	30.2 ± 4.2[b]	7.1 ± 2.0	33.0 ± 6.1	3.2 ± 2.2	2.0 ± 1.4

[a-e]Matched superscripts indicate significant differences at $p < .025$.

Among the aflatoxin-fed Alfin-Slater rats total plasma cholesterol levels were higher than the control values. This difference was more pronounced among those animals fed diet II, as compared to diet I. The cholesterol content of nontumorous liver tissue of aflatoxin-fed Alfin-Slater rats was not significantly different from the cholesterol content of the control livers.

Fatty acid analyses of the various lipid fractions of plasma revealed that, in the plasma sterol ester fraction (Table V) of rats fed diet I, aflatoxin administration results in decreased levels of C18:0 and C18:1 fatty acids. When diet II was fed, however, the C18:1 acid increased markedly in response to aflatoxin. When diet I, supplemented with aflatoxin, was fed, the amount of arachidonic acid (C20:4) was nearly doubled. When aflatoxin was included in diet II, the amount of arachidonic acid decreased by 50%, with a concurrent increase in the C20:3/C20:4 ratio. In view of the low levels of linoleic acid in diet II, the appearance of C20:3 is not unexpected among these animals. The inclusion of aflatoxin in the diet appears to aggravate this condition, possibly by increasing the utilization of arachidonic acid (C20:4). These changes occurred in both strains of animals.

No changes were observed in the fatty acids of plasma triglyceride fraction. However, in the plasma phospholipid fraction, the most striking changes observed in Charles River rats (Table VI) in response to aflatoxin administration were a threefold reduction in the amount of C20:4 acid present, accompanied by corresponding increases in both C18:1 and C18:2 acids sugesting either a possible impairment of arachidonic acid synthesis or perhaps an increased utilization of this fatty acid in response to aflatoxin administration in those rats fed both diets.

The fatty acids of the plasma phospholipid fraction of the Alfin-Slater rats did not react in a similar fashion. When diet I was fed, there were decreases in the levels of C16:0, C18:0, and C18:1 fatty acids as a result of aflatoxin administration, and, when diet II was fed there were decreases in C16:1, C18:0, and C18:1 fatty acids as a result of aflatoxin administration. Although there was very little C20:4 fatty acid present, the levels increased in response to aflatoxin when diet I was fed and decreased when diet II was fed, as was previously observed in the sterol ester fraction.

In all the fractions of liver lipid studied, in both rat strains administered aflatoxin, when compared with diet I, diet II resulted in fatty acid patterns reminiscent of those seen in essential fatty acid deficiency, i.e. increased amounts of C16:1 and C18:1 acids and decreased amounts of C18:2 and C20:4.

In the liver sterol ester fraction (Table VII), aflatoxin administration to both strains of rats resulted in slightly decreased amounts of C16:0 and C18:0 fatty acids and increased amounts of C18:1, regardless of the basal diet. In rats fed diet I, aflatoxin administration resulted in a relative reduction in levels of C20:4. Rats fed diet II without aflatoxin had levels of C20:4 so low that administration of aflatoxin caused no further detectable reduction.

No significant differences were observed in the triglyceride fraction of any of the groups which could be attributed to aflatoxin administration.

In the phospholipid fraction of liver (Table VIII), aflatoxin administration resulted in elevated levels of C18:1 acid in Charles River rats fed diet I or diet II. In addition, among these rats fed diet II, the C20:3/C20:4 ratio nearly doubled when aflatoxin was administered. Under these experimental conditions, it appears that one of the effects of aflatoxin administration to well nourished rats (diet I) is to increase the requirement for essential fatty acids. In rats receiving marginal amounts of essential fatty acids (diet II), a fatty acid pattern similar to an essential fatty acid deficiency pattern was observed which was not exaggerated by the addition of aflatoxin to the diet.

Liver tumors frequently appeared as greyish fatty nodules. Fatty acid analysis of these nodules from aflatoxin-fed Alfin-Slater rats revealed a difference when compared with the surrounding nontumorous liver tissue (Table IX). When diet I plus aflatoxin was fed, it was

TABLE VIII

Major Fatty Acids of the Total Liver Phospholipids of Charles River Rats Fed Two Different Diets with and without Aflatoxin

Group	Fatty acids (%)				
	18:1	20:3[a]	20:3[b]	20:4	20:3/20:4
CA I-18	16.8 ± 1.4[c]	---	Trace	24.3 ± 5.0	---
CC I-18	12.6 ± 0.4[c]	---	Trace	24.5 ± 2.5	---
CA II-18	23.8 ± 4.5[d]	6.4	2.1	13.4 ± 1.7	.48
CC II-18	18.8 ± 0.8[d]	4.4	2.8	16.2 ± 6.3	.27

[a]Δ5, 8, 11.

[b]Δ8,11,14.

[c,d]Matched superscripts indicate significant differences at $p < .025$.

TABLE IX

Fatty Acid Composition of Liver (L) and Liver Tumor (T) of Alfin-Slater Rats Fed Two Different Diets Supplemented with Aflatoxin

Lipid fraction	Group	Fatty acids (%)					
		16:0	16:1	18:0	18:1	18:2	20:4
Sterol ester	AA I-L	11.6 ± 2.7	5.6 ± 1.7	5.6 ± 0.3	39.4 ± 6.6	11.4 ± 1.6	6.9 ± 1.6
	AA I-T	10.6 ± 3.1	10.1 ± 2.4	4.7 ± 1.8	46.6 ± 1.8	10.8 ± 3.5	6.9 ± 2.7
	AA II-L	21.1 ± 2.9	7.8 ± 0.9	6.6 ± 1.4	38.2 ± 1.3	6.0 ± 1.6	2.6 ± 2.0
	AA II-T	15.9 ± 6.4	7.9 ± 1.0	12.2 –	35.0 ± 0	5.6 ± 1.6	8.2 ± 2.0
Triglyceride	AA I-L	18.8 ± 3.2[a]	3.5 ± 1.7	3.9 ± 1.2	35.1 ± 1.3	23.9 ± 3.8	6.2 ± 0.8
	AA I-T	12.7 ± 2.0[a]	6.8 ± 2.0	2.6 ± 2.5	46.8 ± 7.8	15.6 ± 5.1	5.5 ± 2.0
	AA II-L	22.4 ± 3.6[b]	8.9 ± 0.7[c]	6.5 ± 2.1	55.4 ± 0.6	4.7 ± 2.0	Trace
	AA II-T	12.2 ± 1.7[b]	5.4 ± 1.0[c]	7.1 ± 2.9	54.7 ± 9.8	5.2 ± 2.7	Trace
Phospholipid	AA I-L	14.3 ± 4.2	2.0 ± 1.1	26.5 ± 7.6	14.0 ± 1.8[d]	10.6 ± 1.2	21.4 ± 5.2
	AA I-T	18.2 ± 1.8	4.4 ± 1.9	15.2 ± 2.6	23.6 ± 4.6[d]	9.9 ± 3.5	17.3 ± 6.0
	AA II-L	21.7 ± 6.6	4.5 ± 2.1	25.7 ± 6.3	21.1 ± 5.1	5.1 ± 1.1	6.7 ± 6.0
	AA II-T	15.4 ± 2.1	4.0 ± 1.0	13.9 ± 3.0	34.8 ± 7.7	7.1 ± 3.3	12.8 ± 0.9

[a-d]Matched superscripts indicate significant differences at $p<.025$.

observed that, in all lipid fractions of the tumor tissue, there were increased amounts of C16:1 and C18:1 fatty acid accompanied by corresponding decreases in C16:0 and C18:0 acids, suggesting a possible alteration in the enzymes which desaturate C16:0 and C18:0 to their corresponding unsaturated analogues.

Among rats fed diet II plus aflatoxin, the concentration of C20:4 acid was found to be elevated in the sterol ester and phospholipid fractions of tumor tissue as compared with the surrounding tissue. The increased incorporation of this essential fatty acid into tumor tissue may be related to the increased size and severity of the tumors observed in these animals.

This study has demonstrated that Alfin-Slater rats, as well as Charles River rats, are susceptible to aflatoxin toxicity when it is fed at the 1.7 ppm level in the diet. This susceptibility, based upon growth, pathology, and biochemical analysis varies in severity based upon the strain of the rat, as well as the different components of the diet. For example, growth in Charles River rats reflects to a greater extent the type of diet fed and less the aflatoxin content than does growth in Alfin-Slater rats. Liver involvement as a result of aflatoxin administration (both diets) was more extensive in Charles River rats than in Alfin-Slater rats. This was undoubltedly related to the extreme obesity of these animals with subsequent fatty infiltration of the liver. Generally, the livers of rats of both species fed diet I were less affected by aflatoxin than were livers of their counterparts fed diet II.

The type of diet fed also was found to produce changes which affected the animals' response to aflatoxin. Liver size in aflatoxin-fed Alfin-Slater rats was increased by a factor of 5 when diet II was fed, but only by a factor of 2 when diet I was fed. Biochemical data indicate the existence of a condition similar to an essential fatty acid deficiency in those rats fed diet II which seems to affect the response of the animals to aflatoxin. For instance, in Alfin-Slater rats when aflatoxin is fed to rats on diet II, there is a greater increase in plasma and liver cholesterol than when aflatoxin is fed to rats on diet I. Plasma fatty acid patterns also indicate an effect of aflatoxin which is suggestive of essential fatty acid deficiency. Liver fatty acid data, on the other hand, show that aflatoxin produces a pronounced pattern which is similar to essential fatty acid deficiency in rats with normally satisfactory essential fatty acid concentrations in the diet (diet I), and that, when the animal is fed an aflatoxin supplemented diet which is marginally deficient in essential fatty acid (diet II), the biochemical effect is less pronounced but the severity of the tumor seems to be aggravated.

The data obtained in this study suggest that the dietary fat source may exert an effect upon the response of the organism to aflatoxin. Since the two diets tested contained different sources of protein, as well as vitamins and essential minerals, it is also possible that some other component of the diet, or perhaps two or more nutrients acting synergistically, may be a responsible factor in altering the animal's response to the toxin.

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