Dietary Factors and Aflatoxin Toxicity: II. Effect of Fat Source upon Aflatoxicosis in Rats

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ABSTRACT

Previous studies in this laboratory have indicated that the tumorigenic and biochemical response of rats to aflatoxin may be affected by diet. To clarify the possible importance of the fat component of the diet in altering these biochemical responses a cross-over experiment was devised in which peanut oil and lard were reversed in two diets containing different protein sources and vitamin mixtures. It was found that more concentrated doses of aflatoxin administered for a shorter period of time had a more inhibitory effect upon growth, and liver pathology also appeared to be worse than when smaller doses were given for longer periods of time. There were slight differences in pathology due to the basal diet among peanut oil-fed rats, and large differences when the fat component was lard. Plasma cholesterol levels were elevated as a result of the aflatoxin administration, regardless of the diet, and liver cholesterol levels were elevated in response to aflatoxin when diets containing the more varied protein source were fed. In all fractions of plasma fatty acids, fatty acid patterns similar to those observed in marginal essential fatty acid deficiency were seen when lard was fed. The observations that the more restricted protein diet or some component of it, reacts with lard to produce signs similar to those typical of essential fatty acid deficiency and that this regimen produces the most severe liver pathology further establishes the importance of the diet as a means of modifying the response of the organism to aflatoxin.

INTRODUCTION

The acute and chronic effects of aflatoxin and its metabolites have been studied in detail. These effects recently were reviewed by Wogan and Pong (1). It now seems to be well established that aflatoxin exerts dramatic effects upon several phases or normal metabolism. Although dietary manipulation as a means of altering the organisms response to this toxin has been studied by numerous investigators, there seems to be a lack of agreement in several areas. Several investigators have reported that, when the protein level in the diet is low, susceptibility to aflatoxin toxicity is increased (2,3). On the other hand, under different experimental conditions, where low doses of the toxin were fed for longer periods of time, low protein diets were reported to exert a protective effect (4,5). The reason for this discrepancy is not apparent and clearly indicates the need for further work clarifying the role of dietary protein in relation of aflatoxicosis. In addition the quality of the dietary protein must not be overlooked. It has been suggested that low protein diets may exert their effect upon aflatoxicosis by failing to provide needed amino acids in the correct proportions (6). It also has been shown by Newberne, et al., (7) that the addition of certain single amino acids to the diet will alleviate the toxic effects of aflatoxin, while the addition of these amino acids in combination potentiates the toxic effect. Other workers also have reported the potentiating effect, upon aflatoxin toxicity, of adequate levels of a low quality protein diet (8).

Since one of the first symptoms of chronic aflatoxicosis is fatty degeneration of the liver, it is not surprising to find that, as a result of aflatoxin feeding, marked changes occur in the composition of various tissue lipids (9,10). It also has

TABLE I

Diets Used in a Crossover Experiment Utilizing Two Different Levels of Aflatoxin B_1 for Varying Periods of Time

Constituent	Diet I %	Diet II %	Diet III %	Diet IV %
Peanut butter[a]	35.00	--	--	--
Peanut meal[b]	--	17.50	34.00	34.00
Casein	--	--	7.00	7.00
Ground whole wheat	43.50	43.50	--	--
Lactalbumin	5.08	5.08	--	--
Skim milk powder	15.00	15.00	--	--
Salt (NaCl)	1.00	1.00	--	--
Salts mix[c]	--	--	5.00	5.00
Calcium carbonate	.50	.50	--	--
Vitamin mix[d]	--	--	2.00	2.00
α-Tocopherol[d]	.01	.01	--	--
Crystalets (vitamins A, D)[f]	.0015	.0015	--	--
Lard	--	17.50	20.00	--
Peanut oil	--	--	--	20.00
Protein content	24.4	24.4	24.5	24.5
Fat content	18.5	18.6	20.2	20.2

[a]Commercial product obtained from CPC International, Union, N.J.

[b]Defatted commercial product (10)

[c]Wesson modification of the Osborne and Mendel Salt Mix (13)

[d]See ref. 14.

[e]1 mg = 0.25 international units as α-tocopheryl acetate.

[f]500 international units vitamin A and 50 international units vitamin D/mg.

been shown that alterations in dietary fat may affect the toxicity of aflatoxin. Hamilton, et al., suggested that, on both chickens and turkeys, high levels of dietary fat exert a protective effect when aflatoxin is fed (11,12).

Previous studies in our laboratory compared the effects of aflatoxin incorporated into two different diets, upon two different strains of rats (10). The diets contained either a varied natural protein source and peanut oil or a more restricted protein source, a commercial vitamin mixture and lard. The results indicated that there were differences in both the qualitative and quantitative aspects of tumor development and in certain biochemical responses. In general, the livers of the rats fed diets containing a varied natural protein source and peanut oil, were less affected by aflatoxin administration than were those fed a more restricted protein source, lard, and synthetic vitamin mixture. Biochemical analyses indicated that the latter diet produced a lipid profile resembling that seen in essential fatty acid deficiency which seemed to be related to the response of the animal to aflatoxin in that these signs were aggravated when aflatoxin was fed.

This present study was designed to clarify the possible importance of the fat source as opposed to some other component of the diet in affecting these differences. For this purpose, a cross-over experiment was devised in which the two fats to be studied were reversed in the two diets containing different protein and vitamin sources, while the other components were kept constant.

EXPERIMENTAL PROCEDURES

The groups are numbered I-IV depending upon the diet fed, and each consisted of either 10 or 16 male rats of the former USC strain. Aflatoxin B_1 at the 0.36 ppm level was included in those diets designated A. These animals received the aflatoxin containing diets for the full 15 month experimental period. Aflatoxin B_1 at the 0.6 ppm level was included in those diets designated B for 9 months after which these rats were put on the control diets without aflatoxin until sacrificed at 15 months. The total amount of aflatoxin consumed by the animals in this experiment was the same in both A and B groups and was the same as the total amount consumed in previous studies in this laboratory where aflatoxin B_1 was fed at the 1.7 ppm level for 3 months.

The diets used are shown in Table I. Diets I and II are the diets used previously in this laboratory (10). The fat source in diets I and IV is predominantly peanut oil, and that in diets II and III is lard. The peanut oil in diets I and IV contributes 5% linoleic acid as compared to 2% provided by lard in diets II and III. Diets I and II contain peanut meal, whole wheat, lactalbumin, and casein as the protein sources, while, in diets III and IV, casein and peanut meal are the protein sources, and synthetic salts and vitamin mixes are used. The peanut meal of diets II, III, and IV was prepared from the commercially obtained peanut butter of diet I, as previously described (10). The vitamin mix, though adequate for growth, does not contain either para-aminobenzoic acid or biotin.

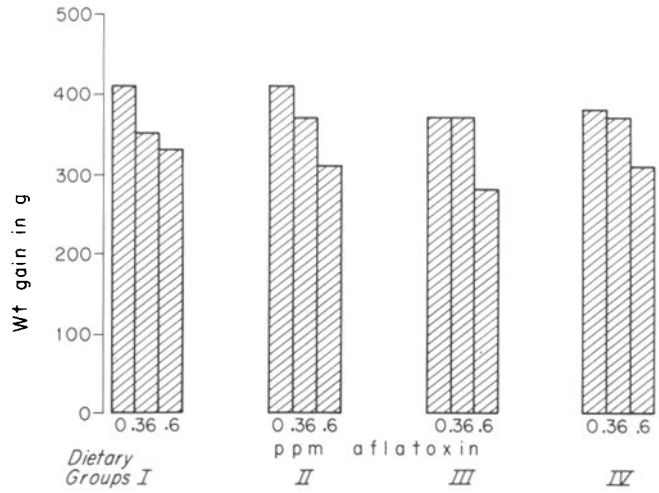

FIG. 1. Summary of wt gain of rats after 15 months on diets.

During the 15 month experimental period, records were kept of growth, morbidity, and mortality. At the time of sacrifice, gross, as well as histopathological, examination of tissues was conducted and organ wt were compared. Biochemical analyses included were by methods which have been described previously (15,16).

RESULTS AND DISCUSSION

Wt gain during the 15 month experimental period is shown in Figure 1. It is evident that the reduced wt gain which results from aflatoxin administration is more pronounced when more concentrated doses of aflatoxin are given for a shorter period of time than when the same total

TABLE II

Summary of Gross Liver Pathology of Rats at Time of Sacrifice after 15 Months on Diets

Group	Number of rats	Liver wt	% of body wt	Normal %	Slight[1] %	Moderate[2] %	Severe[3] %
I	5[4]	11.3 ± 1.1[ab]	2	100	--	--	--
IA	7	14.7 ± 3.3[a]	3	43	43	14	--
IB	7[4]	38.7 ± 14.0[b]	9	--	--	43	57
II	5[4]	12.7 ± 1.5[c]	3	100	--	--	--
IIA	9	14.1 ± 1.1[d]	3	66	33	--	--
IIB	6[4]	36.9 ± 12.2[cd]	9	--	--	66	33
III	4[4]	12.8 ± 0.4[ef]	3	100	--	--	--
IIIA	9	20.1 ± 4.9[eg]	4	11	22	55	11
IIIB	6[4]	42.0 ± 16.4[fg]	13	--	--	17	83
IV	4[4]	12.6 ± 0.4[hi]	3	100	--	--	--
IVA	9	16.9 ± 3.8[hj]	4	33	--	66	--
IVB	5	35.5 ± 14.6[ij]	10	--	20	20	60

[1]Slight involvement—small cysts and nodules (2 x 2 x 2 mm); mild fatty degeneration.

[2]Moderate involvement—few large nodules or cysts (1 x 1 x 1 cm) in otherwise mildly involved livers.

[3]Severe involvement—multiple large and small hepatoma and cysts; severe fatty degeneration of "normal" tissue; irregular texture, etc.

[4]Half of the rats from these groups were sacrificed after 12 months on diets (17).

a,b, etc. Matched superscripts show significant differences between individual groups at $p<.05$.

TABLE III

Plasma Cholesterol, Plasma Tocopherol, and Liver Cholesterol of Rats Sacrificed after 15 Months on Diet

Group	Plasma cholesterol		Plasma tocopherol	Liver cholesterol	
	total (mg %)	% free	(μg/ml)	total (mg/g)	% free
I	85.4 ± 7.1[a]	33	7.5 ± 2.4	2.27 ± 0.21[fg]	78
IA	107.5 ± 12.1[a]	35	10.9 ± 4.7	2.82 ± 0.21[f]	66
IB	127.0 ± 66.0	37	8.6 ± 5.2	2.92 ± 0.23[g]	65
II	94.0 ± 7.5	37	9.2 ± 2.7	1.94 ± 0.16[hi]	86
IIA	101.5 ± 15.8	36	9.9 ± 3.8	2.35 ± 0.20[h]	76
IIB	107.0 ± 17.3	50	10.9 ± 3.0	2.50 ± 0.20[i]	77
III	79.9 ± 22.8[bc]	30	12.1 ± 5.1	3.38 ± 0.76	57
IIIA	134.6 ± 24.9[b]	38	22.3 ± 4.9	5.50 ± 3.05	43
IIIB	172.3 ± 73.9[c]	42	13.4 ± 3.4	3.00 ± 0.40	67
IV	105.8 ± 15.4[d]	32	17.2 ± 4.6	2.20 ± 0.03	75
IVA	132.2 ± 17.6[d]	44	16.3 ± 4.3	2.54 ± 0.65	68
IVB	171.4 ± 57.8	39	21.4 ± 14.0	2.58 ± 0.32	70

a,b,c, etc. Matched superscripts show significant differences between individual groups at $p<.05$.

TABLE IV

Major Fatty Acids (%) of the Sterol Ester Fraction of Pooled Plasma of Rats Sacrificed after 15 Months on Diets

Group	Fatty acids					
	16:0	16:1	18:0	18:1	18:2	20:4
I	9.8	2.4	4.6	15.0	15.4	43.5
IA	10.5	1.8	4.4	14.0	23.4	40.1
IB	11.1	1.8	3.1	13.6	21.4	39.3
II	20.0	9.3	6.7	26.3	16.6	19.2
IIA	8.6	4.8	7.8	20.1	17.1	34.0
IIB	12.8	6.7	3.7	27.8	16.9	24.6
III	9.8	7.2	4.4	22.9	9.8	30.8
IIIA	11.0	8.1	2.4	27.9	14.2	25.9
IIIB	12.4	6.6	3.6	36.5	11.7	17.1
IV	10.5	1.8	4.4	14.0	23.4	40.1
IVA	9.6	1.8	2.6	10.7	18.6	51.6
IVB	10.2	2.1	3.4	10.8	18.6	47.8

amount is administered at a lower level for a longer period of time.

Although, during the course of the experiment, a few more rats died on diets I and II than on diets III and IV, it was observed at autopsy that liver pathology (summarized in Table II) was more severe among those rats fed diets III and IV. Liver pathology was also relatively more severe in those rats receiving the more concentrated doses of aflatoxin. It was alo observed that, whereas those rats receiving peanut oil diets (diets I and IV) had only slight differences in pathology due to the basal diet, i.e. group IB has 51% severe liver involvement and group IVB has 60% severe liver involvement, those receiving lard as the fat source showed much more severe liver involvement with basal diet III than basal diet II, i.e. group IIB had 33% incidence of severe liver involvement, and group IIIB had 83% incidence of severe liver involvement.

Other tissues, i.e. spleen, stomach, heart, aortas, and testes were examined both grossly and histologically and were found to be normal. In several cases, aflatoxin-dosed rats were found to have kidney tumors and necrosis, but, since they appeared sporadically in all the aflatoxin-dosed animals, these could not be ascribed to any particular diet or level of aflatoxin in the diet.

Histologically, liver nodules were found to be composed generally of parenchymatous cells and were characterized by nodular foci of vacuolization, dilated tubules, and cystic areas. Many of the livers were found to have necrotic foci, and some of the tumors were also necrotic. Livers of rats fed diets III and IV exhibited slight to moderate fat phanerosis, and fatty inflitration was observed even in the controls fed these diets.

No significant differences were observed in the wt of spleen, kidney, testes, heart, and adrenals. However, the livers of the animals fed the more concentrated dose of aflatoxin for the shorter time period were more than twice as large as those from rats fed the less concentrated dose for a longer period of time (Table II).

It was also observed that when the fat component of the diet consisted of peanut oil (diets I and IV) the size of the liver after aflatoxin administration was the same, regardless of the basal diet. When the fat component of the diet was lard (diets II and III), however, the livers of rats fed diet III with aflatoxin were larger, both absolutely and as a percent of their total body wt, than were those fed diet II with aflatoxin. While this phenomenon was not statistically significant, it was apparent regardless of the level at which aflatoxin was incorporated into the diet. These observations reflect the aforementioned differences in liver pathology and suggest that perhaps some component of the diet containing the restricted protein source and synthetic vitamin mixture might interact with the fat source (lard) to potentiate the effect of aflatoxin toxicity upon liver pathology.

Plasma cholesterol, plasma tocopherol, and liver cholesterol values are shown in Table III. Samples of nontumorous liver tissue were analyzed for total liver lipid and cholesterol. No significant differences in total lipid content were observed as a result of either basal diet or aflatoxin administration.

It appears that, in all the rats fed aflatoxin, there are increased amounts of cholesterol in liver and plasma and that, the more concentrated the aflatoxin dose, the higher

TABLE V

Major Fatty Acids (%) of the Triglyceride Fraction of Pooled Plasma of Rats Sacrificed after 15 Months on Diets

Group	Fatty acids					
	16:0	16:1	18:0	18:1	18:2	20:4
I	18.0	3.5	7.4	35.3	21.6	6.6
IA	18.0	2.4	6.2	36.4	24.1	8.0
IB	16.2	3.2	5.8	33.7	18.8	3.8
II	25.6	6.1	10.4	38.8	4.3	3.9
IIA	25.2	5.8	7.5	48.0	5.8	2.9
IIB	26.1	7.7	10.4	43.3	5.5	2.1
III	22.1	9.2	6.6	48.2	8.5	1.1
IIIA	22.7	6.9	7.7	51.4	4.2	1.5
IIIB	27.5	9.8	7.2	47.1	4.3	--
IV	17.2	3.4	7.6	29.6	16.5	8.4
IVA	21.0	4.7	5.6	40.1	18.9	6.1
IVB	21.1	3.5	8.5	35.1	15.7	7.7

TABLE VI

Major Fatty Acids (%) of the Phospholipid Fraction of Pooled Plasma of Rats Sacrificed after 15 Months on Diets

Group	Fatty acids							
	16:0	16:1	18:0	18:1	18:2	20:3	20:4	20:3/20:4
I	19.5	1.1	28.4	10.8	19.7	--	15.1	--
IA	24.3	1.2	28.8	12.4	18.9	tr	14.2	--
IB	24.3	1.6	23.4	13.1	18.4	--	13.2	--
II	21.1	1.9	28.4	21.9	15.6	--	9.8	--
IIA	22.9	2.2	25.7	20.8	12.4	1.8	8.8	.20
IIB	23.2	1.6	23.4	22.6	12.5	2.5	8.9	.28
III	18.6	3.0	30.0	21.3	7.0	5.5	6.1	.90
IIIA	21.0	3.3	26.4	25.3	7.5	6.0	7.5	.80
IIIB	23.6	4.6	22.6	29.1	7.4	4.4	4.5	.98
IV	22.6	1.1	26.1	12.4	15.8	tr	16.2	.20
IVA	18.3	2.1	28.2	13.0	17.2	tr	15.0	--
IVB	28.8	1.9	23.9	11.3	14.4	--	15.3	--

are the levels of cholesterol. This could reflect either increased biosynthesis of cholesterol or impairment of cholesterol catabolism. The type of basal diet fed appears to have an effect upon this response to aflatoxin in that rats fed diets I and III concentrate cholesterol in the liver in response to aflatoxin, whereas those rats consuming diets III and IV do not. Among these rats (diets III and IV) marked increases in cholesterol occur in the plasma. The data suggest that when aflatoxin is fed that there may be some impairment of cholesterol transport which is mediated by the basal diet which subsequently affects the distribution of cholesterol. Since there are also differences in pathology, depending upon which basal diet is fed, these findings may be related to tumorigenesis.

Plasma tocopherol levels were found to be elevated uniformly in all groups receiving the basal diet containing the more restricted protein source (diets III and IV). This phenomenon is unexplained in view of the fact that diets III and IV contained only 2.7 mg dl-α-tocopheryl acetate/100 g diet, whereas diets I and II, utilizing the more varied protein source, contained 10 mg dl-α-tocopheryl acetate/100 g diet.

In all fractions of plasma fatty acids (Tables IV, V, and VI), it was observed that, when diets II and III (lard containing) were fed, fatty acid patterns were similar to what one would expect to find in essential fatty acid deficiency, i.e. levels of C16:1 and C18:1 were elevated; levels of C20:4 were reduced. This resemblence to an essential fatty acid deficiency profile was most apparent in the phospholipid fraction, where C20:3 also appeared and, in all fractions, was more pronounced in those animals fed diet III, than among those fed diet II. Some component of diet III appears to act in conjunction with lard to produce this effect.

In the sterol ester fraction of liver (Table VII), decreases in C16:0 and C18:0 and increases in C18:1 were observed in response to aflatoxin among those rats receiving diets containing peanut oil as the fat source (groups I and IV). It also was observed that levels of C18:0 and C20:4 in group III were ca. half those in group II regardless of aflatoxin supplementation. There were no changes in liver triglycerides which could be attributed to either the basal diets or aflatoxin supplementation.

In the phospholipid fraction of liver (Table VIII), a fatty acid profile, similar to that seen in essential fatty acid deficiency, which previously was noted in groups II and III, also was observed regardless of aflatoxin supplementation. Here again, levels of C16:1 and C18:1 were elevated; levels of C18:2 and C20:4 were decreased; and C20:3 appeared.

Comparisons of the fatty acid composition of tumor, as compared to liver lipids, are shown in Tables IX and X. These values represent the ratio of the percent of each fatty acid in tumor lipid to the percent of that fatty acid in liver tissue, i.e. values > 1 indicate accumulation of fatty acid in tumor. In the sterol ester fraction in all groups, except group I, tumor tissue was found to have less C16:0 than the surrounding liver tissue. In both groups getting the diet with the limited protein source (groups III and IV), there was a rather marked accumulation of essential fatty acids in tumor tissue was compared to liver.

No consistent differences were observed in the triglyceride fraction; however, in the phospholipid fraction, it was

TABLE VII

Major Fatty Acids (%) of Liver Sterol Esters of Rats Sacrificed after 15 Months on Diets

Group	Fatty acids					
	16:0	16:1	18:0	18:1	18:2	20:4
I	27.0 ± 8.2	5.1 ± 0.4	11.4 ± 3.3	30.8 ± 6.1	11.0 ± 3.9	6.7 ± 1.3
IA	21.1 ± 4.4	3.7 ± 2.2	7.6 ± 2.6	43.5 ± 3.3	13.1 ± 3.5	6.3 ± 1.7
IB	17.9 ± 1.1	3.2 ± 1.5	9.5 ± 4.1	42.2 ± 8.2	15.5 ± 1.5	8.2 ± 3.8
II	26.8 ± 5.4	6.6 ± 1.2	10.8 ± 2.5	37.3 ± 3.8	7.7 ± 5.4	4.4 ± 2.3
IIA	26.2 ± 0.9	10.2 ± 5.4	13.0 ± 2.9	34.0 ± 4.9	6.2 ± 1.0	4.0 ± 3.5
IIB	22.3 ± 3.1	7.2 ± 0.7	9.8 ± 1.5	42.2 ± 5.4	8.3 ± 2.4	6.6 ± 3.3
III	32.3 ± 7.8	8.4 ± 3.1	12.8 ± 3.7	36.6 ± 5.6	4.7 ± 1.9	3.1 ± 3.0
IIIA	26.5 ± 3.5	10.1 ± 2.4	7.5 ± 2.2	48.2 ± 5.7	2.9 ± 0.6	2.3 ± 1.5
IIIB	32.2 ± 3.6	7.1 ± 1.1	9.6 ± 3.6	40.7 ± 8.2	4.2 ± 1.5	2.7 ± 2.7
IV	37.9 ± 1.6[a]	5.2 ± 1.6	20.7 ± 8.0	26.1 ± 3.8	8.8 ± 1.8	5.3 ± 1.7
IVA	33.9 ± 4.6	4.3 ± 0.9	11.8 ± 3.4	29.5 ± 6.2	10.8 ± 2.1	5.5 ± 1.7
IVB	31.9 ± 4.8[a]	5.6 ± 1.8	11.6 ± 3.6	31.4 ± 9.2	11.1 ± 3.5	6.2 ± 3.8

[a]Matched superscript shows significant difference between groups at $p<.05$.

TABLE VIII

Major Fatty Acids (%) of Liver Phospholipids of Rats Sacrificed after 15 Months on Diets

Group	Fatty acids									
	16:0	16:1	18:0	18:1	18:2	20:3[1]	20:3[2]	20:4	22:6	20:3/20:4
I	15.0 ± 2.4	1.1 ± 0.7	33.2 ± 4.0	12.5 ± 1.2	13.2 ± 2.7	--	--	23.4 ± 4.1	--	--
IA	15.9 ± 2.1	1.3 ± 0.5	29.8 ± 3.5	13.2 ± 1.4	13.7 ± 1.9	--	--	24.0 ± 2.9	--	--
IB	18.5 ± 2.0	1.3 ± 0.6	28.2 ± 1.5	13.4 ± 4.1	13.4 ± 1.2	--	--	23.9 ± 4.2	Trace	--
II	16.9 ± 2.3	2.8 ± 0.8	28.0 ± 4.2	19.2 ± 3.4	8.6 ± 2.1	3.3 ± 1.5	1.5 ± 1.1	16.5 ± 5.4	2.0 ± 1.8	.20
IIA	16.2 ± 1.2	2.5 ± 1.2	27.8 ± 4.5	18.2 ± 2.3	9.0 ± 1.2	2.8 ± 0.5	1.5 ± 0.3	18.5 ± 2.0	1.8 ± 1.2	.15
IIB	18.1 ± 0.8	1.8 ± 0.6	26.8 ± 2.3	17.2 ± 0.9	9.5 ± 1.2	5.9 ± 3.9	1.1 ± 0.3	20.7 ± 2.6	2.3 ± 1.3	.28
III	15.0 ± 1.4	3.4 ± 1.4	26.5 ± 3.3	20.8 ± 4.1	6.4 ± 0.9	4.5 ± 1.7[b]	1.6 ± 0.5	16.5 ± 3.2	3.7 ± 1.1	.27
IIIA	17.4 ± 3.5	3.9 ± 1.0	27.2 ± 3.2	21.5 ± 2.9	5.9 ± 1.0	4.3 ± 2.6[c]	1.3 ± 0.7	14.5 ± 3.2	1.9 ± 1.7	.30
IIIB	16.5 ± 1.0	2.7 ± 0.2	27.4 ± 1.3	22.0 ± 3.4	5.7 ± 0.8	7.6 ± 0.2[bc]	Trace	14.6 ± 1.1	1.5 ± 1.4	.52
IV	16.8 ± 3.9	1.9 ± 2.0	30.4 ± 2.7[a]	13.1 ± 3.4	10.7 ± 1.5	--	Trace	22.3 ± 9.2	1.3 ± 1.6	--
IVA	14.6 ± 1.7	1.1 ± 0.3	28.7 ± 3.7	14.1 ± 3.1	11.2 ± 1.6	Trace	Trace	25.6 ± 1.7	1.8 ± 2.0	--
IVB	16.7 ± 2.3	1.5 ± 1.8	24.6 ± 4.1[a]	13.9 ± 2.4	11.8 ± 1.3	Trace	Trace	25.6 ± 3.5	1.6 ± 1.3	--

a,b,etc. Matched superscripts show significant differences between individual groups at $p<.05$.

[1]$\Delta 5,8,11$.

[2]$\Delta 8,11,14$.

TABLE IX

Ratios of the Sterol Ester Fatty Acids of Tumor to Sterol Ester Fatty Acids of Liver Tissue of Rats Sacrificed after 15 Months on Diets

Group	Fatty acids					
	16:0	16:1	18:0	18:1	18:2	20:4
IA	0.92	0.92	1.49	0.81	1.09	1.04
IB	1.06	1.96	1.35	0.79	0.89	1.28
IIA	--	--	--	--	--	--
IIB	0.70	0.92	1.06	1.14	0.93	0.71
IIIA	0.69	1.11	0.93	1.09	1.67	1.45
IIIB	0.05	1.29	1.07	0.95	1.85	2.22
IVA	0.59	0.84	0.74	1.41	1.01	2.00
IVB	0.55	0.86	0.91	1.25	1.32	1.54

observed that, with the exception of group I, there were increased levels of C16:0, C16:1, C18:1, and decreased amounts of C18:0 in tumor tissue as compared to the surrounding liver tissue. In groups II and III, where C20:3 ($\Delta 5,8,11$) appeared, levels of this fatty acid in the tumor tissue were one-half to one-third of those in the liver.

The fact that the toxic effect of aflatoxin was less pronounced, both pathologically and biochemically when smaller amounts of aflatoxin were fed for extended periods of time, but was still equal in amount to the total consumed over the shorter periods, lends further support to the hypothesis that there may be a level at which animals can tolerate aflatoxin in their diets without adverse effects.

The observation that diet III, or some component of that diet, reacts (or interacts) with the lard fat source to produce signs similar to those of essential fatty acid deficiency, coupled with the observation that, on this regimen, the rats demonstrated the most severe liver pathology, further established the importance of the diet as a means of modifying the response of the organism to aflatoxin. In addition to the fat, there are as yet unidentified factors which probably mediate or enhance the effects of aflatoxicosis.

TABLE X

Ratios of Phospholipid Fatty Acids of Tumor to Phospholipid Fatty Acids of Liver Tissue of Rats Sacrificed after 15 Months on Diets

Group	Fatty acids						
	16:0	16:1	18:0	18:1	18:2	20:3[a]	20:4
IA	1.17	0.69	0.79	1.04	1.11	--	0.92
IB	0.97	1.64	0.78	1.29	1.09	--	0.94
IIA	--	--	--	--	--	--	--
IIB	1.12	1.78	0.74	1.29	1.10	0.25	0.92
IIIA	1.20	1.85	0.74	1.41	1.47	0.58	0.66
IIIB	1.23	1.64	0.70	1.29	1.29	0.61	0.92
IVA	1.42	2.27	0.75	1.14	1.14	--	0.89
IVB	1.12	2.08	0.88	1.37	1.03	--	0.84

[a]$\Delta 5,8,11$.

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