Effect of Dietary Fat upon Aflatoxicosis in Rats Fed Torula Yeast Containing Diet

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ABSTRACT

This study was designed to study the possible interrelationships between Torula yeast, vitamin E, and the dietary fat source on aflatoxin-induced tumors. Rats were fed Torula yeast-containing basal diets which included 1.7 ppm aflatoxin B_1 with either lard, corn oil or no fat, and with or without vitamin E supplements for 3 months. Thereafter, the respective diets without aflatoxin were fed for ca. 9 months. Animals receiving the vitamin E-deficient diets had a high mortality. Although the vitamin E-deficient, aflatoxin-treated rats had lower wt gains than did the vitamin E-deficient controls, they lived twice as long. In addition, regardless of the dietary fat source, the kidneys and adrenals of these vitamin E-deficient, aflatoxin-supplemented rats were found to be significantly heavier than the controls, and plasma cholesterol levels were elevated. Increased amounts of liver lipid were observed in response to aflatoxin in both corn oil-fed and fat-deficient rats. No such differences were observed in the responses of the vitamin E-supplemented groups to aflatoxin. On the corn oil diet, aflatoxin administration resulted in an increased deposition of polyunsaturated fatty acids in cholesteryl ester and phospholipid fractions in livers of vitamin E-deficient rats and the phospholipid fraction of vitamin E-sufficient rats. The vitamin E-deficient rats exhibited necrosis of the liver, which was alleviated when aflatoxin was included in the diet, and calcification of the kidneys, which was potentiated by the dietary aflatoxin. No tumors were observed in these animals. In animals maintained on vitamin E-sufficient diets for 1 year, growth was depressed as a result of aflatoxin administration with the greatest depression occurring in the group fed corn oil. Spleen wt were decreased in all groups given aflatoxin. However, there were no changes in either plasma or liver cholesterol or total liver lipids which could be attributed to aflatoxin administration. When aflatoxin was fed with lard, the cholesteryl ester, triglyceride, and free fatty acid fractions of plasma had decreased amounts of the C20:4 acid. In the cholesteryl ester fraction only, this change was accompanied by increased levels of C16:0, C18:0, and C18:1 acids. In the liver phospholipids, there were increased levels of mono- and polyunsaturated fatty acids and decreases in the saturated fatty acids. All of the animals receiving aflatoxin exhibited severe necrosis and tumor formation in the kidneys; the animals fed lard had the highest level of involvement and those in the fat-free group the least. Liver pathology was the least marked among the rats fed the fat-free diet. Since aflatoxin-induced tumors are rich in lipids, the fat-free diet may be protective to the animal.

INTRODUCTION

Aflatoxicosis has been observed in a wide range of animal species (1,2), and, in at least three (rat, rainbow trout, and duckling), carcinogenicity has been demonstrated (3-5). It also has been recently implicated as a possible etiological factor in human liver disease in areas where human foodstuffs have been demonstrated to be subject to contamination with the fungus *Aspergillus flavus* (6,7).

At the present time, the prevention of aflatoxicosis is by avoidance of contaminated foodstuffs. Because of the nature of the feed distribution for livestock and the critical shortage of food supplies for human populations in many parts of the world, this avoidance is difficult. A more practical approach to the problem is to determine whether there are dietary additives or modifications which could increase either the tolerance or provide resistance to aflatoxin toxicity.

In a number of instances, dietary modifications have been reported to alter the response of experimental animals to aflatoxin. For instance, diets low in lipotropes (8), as well as cirrhotic diets (9), have been reported to enhance the toxic effects of aflatoxin. Varying the protein level in the diet also has been reported to affect susceptibility to aflatoxin; diets containing low levels of protein, under certain experimental conditions, have produced more severe acute liver pathogenesis in response to aflatoxin administration (10). Studies by other investigators concerning the effects of protein quality upon susceptibility of animals to aflatoxin toxicity have indicated that aflatoxins have a more potent effect when Torula yeast is included in the diet as the sole protein source (11). Under these conditions, Torula yeast has a liver-stressing effect which results in severe necrosis of the liver and eventually death (12,13). Torula yeast is deficient in the sulfur-containing amino acids, vitamin E, and selenium (14,15); the addition of vitamin E to Torula yeast rations will alleviate the liver stressing effect of the Torula yeast completely (12).

In addition, previous work in our laboratory suggests that responses to aflatoxin may be modified by changing the type of dietary fat (16). The present study was designed to determine the effect upon aflatoxin toxicity of a low quality protein, such as Torula yeast, fed with and without vitamin E and with different types of dietary fat.

METHODS

The plan of the experiment is shown in Table I. The fat source is designated either L (lard), Co (corn oil), or F (fat-free). Aflatoxin-supplemented and control groups are designated either A or C, respectively, and the presence of dl-α-tocopheryl acetate, at a level of 0.01% of the diet, is

TABLE I

Plan of Experiment

Group[a]	Fat source	Aflatoxin (ppm)
LC	Stripped lard	0
LA		1.7
LC-E	Stripped lard + vitamin E	0
LA-E		1.7
CoC	Stripped corn oil	0
CoA		1.7
CoC-E	Stripped corn oil + vitamin E	0
CoA-E		1.7
FC	Fat-free	0
FA		1.7
FC-E	Fat-free + vitamin E	0
FA-E		1.7

[a]See text for definition of groups.

TABLE II

Diets

Nutrient	Groups[a] L (%)	Co (%)	F (%)
Torula yeast	30	30	30
Sucrose	61	61	66
Lard, stripped	5	--	--
Corn oil, stripped	--	5	--
Salt mix[b]	4	4	4
Vitamin mix[c]	.25	.25	.25

[a]See text for definition of group.

[b]Wesson modification of Osborne-Mendel formula (17).

[c]Vitamin mix supplied the following in mg/100 g diet: p-aminobenzoic acid, 50; inositol, 50; ascorbic acid, 100; thiamin, 5; Ca pantothenate, 8; niacin, 10; B_{12} triturate, 6; riboflavin, 3; pyridoxine, 3; crystalets (500 international units vitamin A and 50 international units vitamin D/mg), 5; folic acid, 1; menadione, 5; biotin, 0.2.

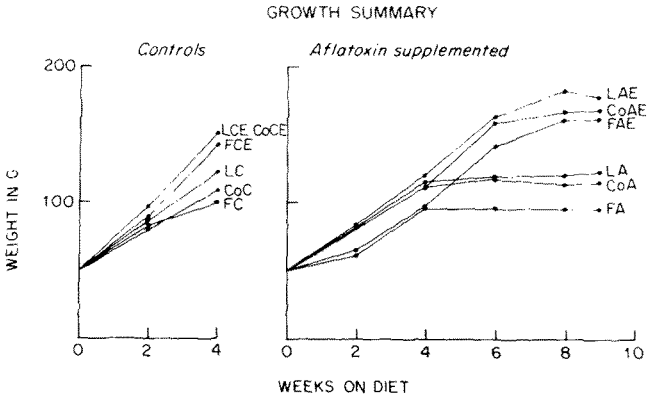

FIG. 1. Growth summary of groups of rats on various diets sacrificed after 4 and 10 weeks on diets. See text for definition of groups.

indicated by E. Pure, crystalline, synethetic aflatoxin B_1 was administered at the 1.7 ppm level for 3 months to male, weanling rats of the former USC strain, after which the animals were maintained on control diets without aflatoxin for 9 months. At the beginning, all groups included 10 rats, however, high mortality (50%) among the vitamin E-deficient rats forced their premature sacrifice after 4-10 weeks on the diets. At this time, half of the vitamin E-supplemented rats also were sacrificed for purposes of comparison.

The diets used (Table II) were similar to those used by other investigators in studies utilizing Torula yeast (1,2) but differ in that the salt and vitamin mixtures are those normally used in our laboratory, since the vitamin mixture used by other investigators proved to be inadequate for the health and well-being of our animals.

Growth and mortality throughout the experimental period were observed and recorded. At the time of sacrifice, the various organs were weighed and examined grossly for pathology and slices of normal tissues (liver, heart, spleen, kidneys, testes, and stomach), as well as tumors, were subjected to histopathological examination. Lipid extracts of plasma and liver were analyzed for cholesterol (18), and total lipid content of liver was determined gravimetrically. The extracted lipids of plasma and liver were separated into three major fractions using thin layer chromatography, transmethylated, and subsequently analyzed utilizing gas liquid chromatography, as has been described previously (18).

RESULTS

Wt gains of the animals achieving 50% mortality between

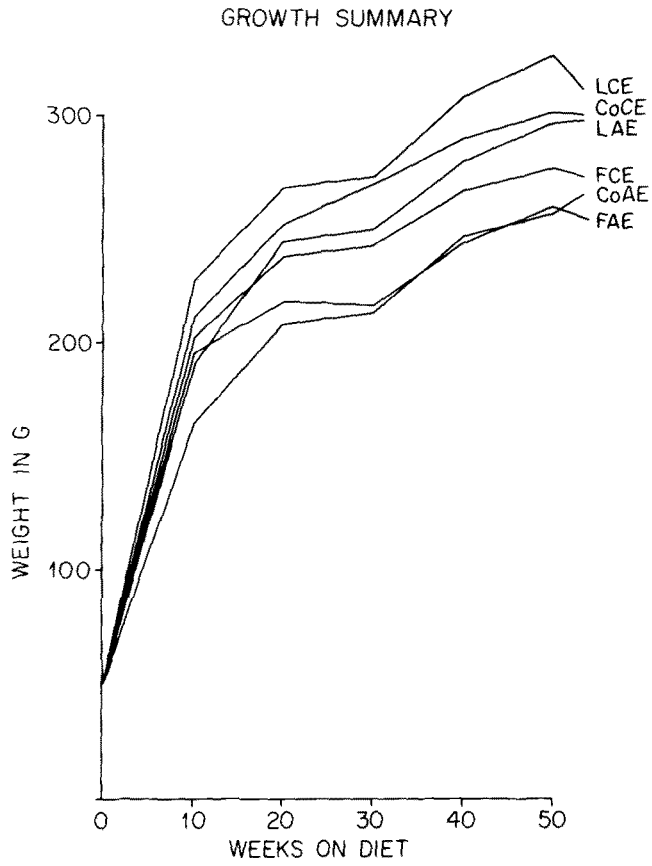

FIG. 2. Growth summary of rats on various diets sacrificed after 1 year. See text for definition of groups.

4-10 weeks of age and their vitamin E-supplemented counterparts are shown in Figure 1. Surprisingly, the vitamin E-deficient, aflatoxin-supplemented rats took twice as long to reach 50% mortality as did the vitamin E-deficient animals which received no aflatoxin. At present, we have no explanation for this phenomenon. In all groups receiving vitamin E in the diet, growth was markedly better than in the rats fed vitamin E-deficient diets, and there was no mortality even when aflatoxin was present in the diet.

The rats which were continued on vitamin E-supplemented diets up to 1 year, exhibited growth patterns (Fig. 2) which were different from those observed at the time of earlier sacrifice. As before, aflatoxin-supplemented animals had poorer wt gains than did the controls. Diets containing corn oil resulted in the most pronounced wt differences between control and experimental animals. In fact, these rats when exposed to aflatoxin showed no better growth than did those rats fed the fat-free diet.

Histopathological examination of the tissues of rats sacrificed early in the experiment revealed varying degrees of necrosis of the liver among the vitamin E-deficient rats. This was expected from the Torula yeast feeding; however, the necrosis was not as severe when aflatoxin was included in the diet which may be responsible for the increased longevity of these animals. There was no liver necrosis among any of the vitamin E-supplemented rats sacrificed at this time. Aflatoxin administration failed to produce tumors in these animals, since the time of exposure to the experimental diets was very short.

Slight to moderate calcification of the kidneys and necrosis and atrophy of the testes were noted among the vitamin E-deficient, nonaflatoxin-supplemented animals; these effects were enhanced by the presence of aflatoxin in the diet. None of the groups of vitamin E-sufficient rats exhibited histological abnormalities in kidneys or testes, whether or not aflatoxin was administered. Other tissues

which were examined but demonstrated no pathology included spleen, stomach, adrenals, hearts, and aortas.

Histopathological examination of the tissues of the vitamin E-supplemented rats which were sacrificed after 1 year clearly showed severe kidney involvement, e.g. necrosis and tumors, in all aflatoxin-fed animals; the rats fed lard had the most extensive involvement, whereas those on fat-free diets the least. It was also apparent that liver tumor formation was least severe among those animals fed the fat-free diet. Although data are not available concerning the efficiency of absorption of aflatoxin when it is fed in very minute quantities, it is possible that the presence of fat in the diet may enhance the absorption of aflatoxin from the gut.

In Table III, it can be seen among the vitamin E-deficient rats that the wt of kidneys and adrenals were significantly greater, both absolutely and relatively, in the aflatoxin-supplemented rats. This finding may be due to the increased degree of calcification in the kidneys and to the added stress imposed on the adrenals during active detoxification of the aflatoxin. This reponse to aflatoxin was not observed in the animals receiving vitamin E which were killed either at the same time as the vitamin E-deficient rats or after 1 year on the diets. The vitamin E-sufficient rats which were sacrificed at 1 year all had smaller spleens, both absolutely and relatively, as a result of aflatoxin supplementation regardless of diet.

Of particular interest is the fact that none of the aflatoxin supplemented rats in this study had enlarged livers. Ordinarily, among rats of this strain, aflatoxin administered at 1.7 ppm for a similar length of time produces livers which are 2-4 times normal size. This increase in size is a reflection of the severity of tumor formation. In this study, livers were not enlarged and tumor formation in the livers not as extensive as has been observed in previous aflatoxin studies in our laboratory (16,18,19). The reason for this is not clear, although it appears that the poor quality protein used in the diets might be responsible.

Among the vitamin E-deficient rats, plasma cholesterol levels were elevated significantly when aflatoxin-supplemented diets were either fat-free or contained lard as the fat source (Table IV). In the corn oil-fed animals, no elevations in plasma cholesterol were observed. Rats fed diets supplemented with both vitamin E and aflatoxin and sacrificed at the same time as the vitamin E-deficient aflatoxin-supplemented animals did not have elevated plasma cholesterol levels.

Increased total lipid concentrations in the liver were observed in response to aflatoxin when animals deficient in vitamin E were fed corn oil diets (Table IV). In addition, there were no marked changes in liver cholesterol levels in any group which could be attributed to either aflatoxin feeding or vitamin E supplementation.

In the rats fed vitamin E-supplemented diets sacrificed after 1 year, there were no changes in plasma or liver cholesterol or in total liver lipid levels in response to aflatoxin administration. Although the total liver lipid in the animals on vitamin E-deficient diets with aflatoxin were higher than those fed the ration without aflatoxin, the liver lipids in all of these groups were still lower than what is normally observed in our rats fed a stock laboratory ration, e.g. ca. 40 mg/g (18). Since the liver is normally capable of synthesizing lipid in large quantities, it may be that some component of the Torula yeast basal ration interferes with some aspect of lipid metabolism, such as fatty acid biosynthesis and transport, and this, in turn, may play a role in the response of the vitamin E-deficient rat to aflatoxin.

In Table V are shown the fatty acid patterns in the cholesteryl ester fraction of livers of the vitamin E-deficient animals. Among those rats fed either the fat-free or lard diets, few changes occurred as a result of the aflatoxin administration. However, when corn oil was fed, aflatoxin administration resulted in increased content in liver of C18:0 and C20:4 and a corresponding decreased concentration of C16:0. These changes were not observed in any of the vitamin E-supplemented rats.

TABLE III

Organ Wt of Vitamin E-Deficient Rats Sacrificed at 50% Mortality

Group[a]	Kidney		Adrenals	
	g	As % body wt	mg	As mg % body wt
LC	1.20 ± .30	0.9	17.8 ± 7.9	14
LA	1.80 ± .21	1.5	39.1 ± 15.0	31
CoC	1.21 ± .24	1.1	22.2 ± 6.7	21
CoA	1.90 ± .60	1.6	32.1 ± 3.1	27
FC	1.09 ± .23[b]	1.1	16.7 ± 6.8	17
FA	1.52 ± .32[b]	1.6	26.8 ± 2.0	28

[a]See text for definition of groups.

[b]All values from A-treated animals are significantly different from controls, except for kidneys in the fat-free group.

TABLE IV

Plasma Cholesterol and Total Liver Lipids in Vitamin E-Deficient Rats Sacrificed at 50% Mortality

Group[a]	Plasma cholesterol mg/100 ml	Total liver lipid mg/g
LC	44.6 ± 5.1[b]	30.8 ± 6.3
LA	69.6 ± 13.4	31.3 ± 6.1
CoC	57.2 ± 5.0	11.0 ± 8.4[b]
CoA	61.9 ± 5.4	26.4 ± 4.4[b]
FC	56.6 ± 9.3[b]	15.9 ± 8.3
FA	73.9 ± 14.8[b]	24.6 ± 12.6

[a]See text for definition of groups.

[b]Superscripts indicate significant differences at $p<.05$.

TABLE V

Major Fatty Acids of Liver Cholesteryl Esters of Vitamin E-Deficient Rats Sacrificed at 50% Mortality

Group[a]	Fatty acid (%)			
	16:0	18:0	18:1	20:4
LC	25.6 ± 2.0	18.0 ± 3.5	23.9 ± 2.7	8.9 ± 7.1
LA	24.7 ± 4.0	14.1 ± 5.4	25.6 ± 6.8	13.0 ± 2.4
CoC	34.6 ± 3.4[b]	7.7 ± 0.5[b]	25.2 ± 6.6	4.3 ± 4.4[b]
CoA	27.3 ± 2.2[b]	16.4 ± 4.3[b]	19.9 ± 2.6	13.0 ± 4.5[b]
FC	25.6 ± 1.0	15.5 ± 4.0	21.8 ± 6.2	14.9 ± 3.9
FA	22.6 ± 4.6	14.1 ± 5.9	30.8 ± 7.0	8.5 ± 6.9

[a]See text for definition of groups.

[b]Superscripts indicate significant differences at $p<.05$.

TABLE VI

Major Fatty Acids of Liver Phospholipids of Vitamin E-Deficient and Sufficient Rats Sacrificed at 50% Mortality and Vitamin E-Sufficient Rats Sacrificed after 1 Year

	Fatty acid (%)					
	Vitamin E-deficient			Vitamin E-sufficient		
Group[a]	18:0	18:2	20:4	18:0	18:2	20:4
LC	21.9 ± 3.0	9.4 ± 1.2	23.4 ± 2.1	22.8 ± 1.4	9.8 ± 1.8	25.6 ± 1.1
LA	21.5 ± 0.6	10.7 ± 7.0	23.8 ± 0.8	24.6 ± 2.2	10.7 ± 0.3	26.1 ± 2.8
CoC	25.2 ± 2.7	12.1 ± 1.1[b]	18.3 ± 6.4	38.8 ± 4.4[b]	10.5 ± 3.6	14.1 ± 2.8[b]
CoA	21.2 ± 2.1	15.2 ± 1.3[b]	26.2 ± 3.7	23.2 ± 1.4[b]	14.1 ± 0.9	25.8 ± 3.4[b]
FC	21.4 ± 0.8	10.7 ± 1.5	19.8 ± 3.2	18.9 ± 4.2	8.1 ± 0.3	18.3 ± 5.5
FA	21.2 ± 3.0	10.0 ± 0.8	19.1 ± 3.6	20.6 ± 1.8	9.9 ± 1.3	26.0 ± 0.8
FC 1 year	--	--	--	27.6 ± 7.4	6.8 ± 2.5[b]	14.5 ± 4.3[b]
FA 1 year	--	--	--	19.0 ± 1.5	9.8 ± 1.8[b]	23.0 ± 4.1[b]

[a]See text for definition of groups.

[b]Superscripts indicate significant differences at $p<.05$ within pairs.

TABLE VII

Major Fatty Acids of Pooled Plasma of Rats Fed Lard-Containing Diets and Sacrificed after 1 Year

	Fatty acid (%)					
Group[a]	16:0	16:1	18:0	18:1	18:2	20:4
Cholesteryl esters						
LC	9.2	10.0	1.6	14.2	18.1	35.9
LA	15.1	12.4	4.2	30.4	14.2	17.8
Triglycerides						
LC	19.7	6.3	4.1	33.3	10.6	9.3
LA	21.4	6.0	5.6	41.2	19.0	2.7
Free fatty acids						
LC	22.9	6.2	8.4	30.9	12.3	10.2
LA	20.5	6.9	6.8	33.3	15.4	2.9

[a]See text for definition of groups.

There were no changes in the composition of the fatty acids of liver triglycerides as a result of aflatoxin administration. However, in the phospholipid fraction of liver lipids (Table VI), of the vitamin E-deficient and vitamin E-sufficient rats, it was observed that, when corn oil was fed, aflatoxin administration resulted in increased deposition of linoleic (C18:2) and arachidonic (C20:4) acids and a decrease in stearic acid (C18:0).

There was also a slight, albeit insignificant, increase in the amount of arachidonic acid in liver phospholipids of animals on the fat-free, vitamin E-supplemented diets as a result of aflatoxin feeding. Since the groups exhibiting increased levels of arachidonic acid also had increased levels of total liver lipid, this increase in polyunsaturated fatty acid content of the liver would appear to be the result of increased deposition of polyunsaturates in the liver, as well as the shifts in their relative concentration.

At the end of 1 year, in the rats fed the vitamin E-supplemented diets, aflatoxin-induced changes in the fatty acids occured only in the phospholipid fraction of liver (Table VI) of rats fed the fat-free diet and consisted of increased levels of arachidonic acid. As also was observed in the animals killed earlier, arachidonic acid was nearly twice as high in the aflatoxin-supplemented group as in the control group.

Among the animals killed after 1 year, changes also were seen in the composition of plasma fatty acids which were not observed in the younger animals. Rats fed lard-containing diets supplemented with aflatoxin had markedly increased levels of oleic acid (C18:1) in the cholesteryl ester and triglyceride fractions and markedly decreased levels of arachidonic acid (C20:4) in the cholesteryl ester, triglyceride, and free fatty acid fractions, indicating, perhaps, an aflatoxin-induced inhibition of enzyme systems which are responsible for the elongation or desaturation of linoleic acid (Table VII).

The high incidence of kidney lesions observed in this study is probably the result of a possible interaction between the dietary protein of poor biological value with aflatoxin.

This study has demonstrated that, under different dietary conditions, there is an altered response, both in terms of mortality and biochemistry, to aflatoxin administration. In addition to the aforementioned variations in lipid metabolism of rats maintained on the experimental diets for a year, in short term studies where the animal is actively engaged in aflatoxin detoxification, aflatoxin appears to be associated with an unexplained lowering of the liver necrotizing effect of the vitamin E-deficient, Torula yeast rations which may, in turn, be associated with the increased longevity of these rats. At the same time, the observed changes in the various lipids of plasma and liver in response to aflatoxin administration, suggest that the quality of the dietary protein is of critical importance in establishing the response of an animal to aflatoxin and also that interactions between the fat and protein sources in the diet can alter the animals' susceptibility to dietary contaminants, such as aflatoxin.

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