Studies of Long Term Administration of Aflatoxin to Rats as a Natural Food Contaminant [1]

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Abstract

The effect of feeding aflatoxin, as a natural food contaminant, to rats over long periods of time was studied using multigeneration and longevity tests. The test animals in the multigeneration study consisted of three groups of rats fed diets containing 0, 1 and 10 ppb of aflatoxin (predominantly B_1) continued over four generations, with animals of the first and fourth generation fed the diets for 104 weeks. These diets were in proper nutritional balance and included 35% ground roasted peanut products; the ration with 0 ppb aflatoxin excluded the peanuts usually discarded; the one with 1 ppb had the roasted discards returned, while the ration with 10 ppb included the discards in amount 10 times that which had been initially removed. Another longevity study was also performed in which rats were fed diets containing aflatoxin at a level of 80 ppb. In this case, the test peanuts, also fed as a simulated peanut butter at 35% concentration, consisted entirely of roasted peanut discards. Control diets provided no peanut components. Animals fed the low levels of aflatoxin grew as well and actually had a higher percentage survival at 104 weeks than did the animals on the control, aflatoxin-free diets. Organ weights, liver total lipid and cholesterol levels were comparable in all groups. Pathological abnormalities, e.g., hemorrhagic and opaque spots and mottling in some of the livers, were attributed to the aging process since the abnormalities appeared in the control as well as the experimental groups. In the animals fed the aflatoxin at 80 ppb, which has been reported by several investigators to produce well-defined hepatomas in rats, there was liver involvement and some biochemical changes occurred that were not noted in the controls. However, no hepatomas were observed in these animals even after 21 months on this diet. The liver lesions, indicative of a toxic effect, have not been associated with the development of hepatomas. It is possible that some components of the diet used in these experiments may have protected the animal against hepatoma formation. Our studies indicate that there may be a tolerance for aflatoxin as judged by results in one species of rats when whole ground roasted peanuts provide the natural contaminant.

Introduction

One of the most potent food toxins to be discovered in recent years is the fungal metabolite, aflatoxin, produced by the mold *Aspergillus flavus*. In 1960 a mysterious Turkey X disease, which decimated a large portion of the turkey crop in Great Britain (1,2) was found to be associated with the groundnut meal (peanut meal) of the ration (3); the cause of the disease was soon established to be the presence of the toxic compound aflatoxin, with which the meal was contaminated (4). The *Aspergillus flavus* mold is one of the most common storage molds. It is found in the soil and in the air throughout the world. Although not all of the *Aspergillus flavus -oryzae* species produce aflatoxin, many of them do. Some species produce aflatoxin B_1 which is more toxic than the aflatoxin G_1 which is usually also formed by these same species. Related forms, e.g., aflatoxin B_2 and G_2, can also be produced under certain conditions and are less toxic (5).

Most animal species are susceptible to aflatoxicosis, with the duckling particularly sensitive, turkey poults and chickens slightly less so, rats slightly more resistant, and sheep remarkably resistant to the effects of aflatoxin toxicity (6). Sublethal doses of the toxin have been shown to be carcinogenic for a number of species. It has been shown that the duck (7) guinea pig (8) and, in particular, the rat respond to the administration of low levels of aflatoxin, either as contaminated peanut meal or as the purified aflatoxin B_1, by the production of liver tumors (10). Various control measures have already been applied and more are being proposed to protect human food supplies from aflatoxin contamination.

The problem of aflatoxicosis becomes a public health problem when one considers the use of peanut meal as protein supplements for undernourished populations (11). It becomes necessary to determine whether there can be low levels of aflatoxin contaminants in food which may possibly be safe for human consumption. Since direct experiments on man are not feasible, experiments on animals must be made which can then be extrapolated to human populations. With this idea in mind we undertook a long term feeding experiment with rats to determine the effects of feeding low levels of aflatoxin as a natural food contaminant.

Experimental Procedures

The plan of our experiment was as follows:

Group A_0 was given a semipurified diet containing 35% peanut butter (0 ppb aflatoxin).

Group A_1, as A_0 but a simulated peanut butter differing in that it contained, as a deliberate contaminant, the roasted peanut discards that are initially removed (1 ppb aflatoxin).

Group A_{10}, as A_0 but a simulated peanut butter differing in that it contained, as a deliberate contaminant, 10 times the roasted peanut discards that are initially removed (10 ppb aflatoxin).

Group A_{80}, semipurified diets containing 35% of another simulated peanut butter, this time made entirely with peanut discards (80 ppb aflatoxin).

Group C_0, semipurified diet containing no peanut components (0 ppb aflatoxin).

The complete composition of the diets is shown in

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TABLE I
Diets

Constituent	Per cent
Groups A_0, A_1, A_{10}, A_{30}	
Peanut butter or simulated peanut butter[a]	35.00
Ground whole wheat	43.40
Lactalbumin	5.0585
Skim milk powder	15.00
Sodium chloride	1.00
Calcium carbonate	0.50
α-Tocopherol acetate	0.04
Crystalets (Vit. A & D)	0.0015
Group C_0	
Ground whole wheat	59.7
Sodium chloride	1.0
Skim milk powder	23.3
Margarine oil (predominantly liquid soybean oil)[b]	16.0

[a] Peanut discards, when included in the diet, were added at the expense of peanut butter.
[b] Contains 4500 USP units of Vit. A and 650 USP units of Vit. D per 100 g.

Table I. These were in proper nutritional balance. The levels of aflatoxin used were established as a result of observing aflatoxin levels in the monthly composites of roasted peanut discards obtained over a two year period from several peanut processing plants throughout the country. The method of analysis was the AOAC Official, First Action, Celite Method (12). The animals fed the diet containing aflatoxin at 80 ppb (predominantly B_1 and calculated to a B_1 equivalent) were given a ration where the peanut component consisted entirely of roasted peanut discards. [Their analyses provided the following values for the aflatoxin components; B_1 equal to 165 ppb; B_2, 96 ppb; G_1, 112 ppb; and G_2, 89 ppb. There were calculated to a B_1 equivalent by multiplying the respective concentrations by a factor relating the recognized (5) seven-day LD_{50} duckling values for B_1 to that for each of the other aflatoxins. The respective LD_{50} values used in our calculations were B_1, 18.2 μg; B_2, 84.8 μg; G_1, 39.2 μg; and G_2, 172.5 μg.] In all cases the simulated peanut butters, developed with controlled degrees of contamination for the present investigations, contained 92.5% of ground roasted peanuts; these were fed at the high level of 35% by weight of the total diet. The control diet C_0 had no peanut components and was one that had been used successfully for more than 10 years in our laboratories in longevity and multigeneration studies with rats.

Using fairly large groups of animals (24 animals per group) we conducted multigeneration and longevity studies. The multigeneration study (Experiment A) was done on groups of animals continuously fed diets containing 0, 1 and 10 ppb of aflatoxin (predominantly B_1 and calculated to a B_1 equivalent) continued over four generations with animals of the first and fourth generations fed the diets for 104 weeks. Another longevity study (Experiment B) was performed on groups of animals fed diets containing 80 ppb of aflatoxin; these animals were killed after 12, 18 and 21 months on the experimental diet.

Results and Discussion

Both male and female rats fed the aflatoxin at 1 and 10 ppb grew as well as the control animals on the peanut product containing no aflatoxin and the control group containing no peanut components. Since more than a 75% mortality was reached before 104 weeks in the fourth generation females fed the C_0 diet, this group was terminated at 89 weeks (Table II).

The longevity and mortality data revealed that among the rats fed the low levels of aflatoxin both males and females had a higher percentage of survival at 104 weeks than the animals on the 0 aflatoxin diets, i.e., the A_0 or on the non-peanut control diet, C_0 (Table III). The survival was better in the peanut product-fed groups than in the C_0 groups, better in the A_1 than either the A_0 or the A_{10} groups, and slightly better in the fourth than the first generations. At the time that they were killed, organ weights were comparable in all groups.

Reproduction and lactation data are shown in Table IV. The number of successful pregnancies over three generations is very similar in all groups studied and there are no significant differences in the number of young survivors or in the weight of the weanling rat regardless of the amount and length of exposure to the 1 and 10 ppb of aflatoxin in the diet.

Liver weights, liver lipids and liver cholesterol values were also similar in most cases (Table V). The peanut product-fed male animals seemed to have higher total lipids and higher liver cholesterol levels in the fourth generation than did the non-peanut butter controls. However, this could not be attributed to the aflatoxin content of the diet since the A_0 animals had values similar to the A_1 and A_{10} groups. No such differences were observed in the liver values of the female of the species.

Since the liver is evidently the first and primary organ to show pathological changes resulting from aflatoxin toxicity, this report will be restricted mainly to those changes which occurred in the liver. However, in addition to the liver, microscopic sections were prepared routinely on pituitary, adrenals, gonads, thyroid, stomach, kidney, lung, internal genitalia and mammary glands. No tumors were present in the livers of the animals in any of the groups. There were visible tumors in some animals in the pituitary, adrenals and thyroid glands. Since

TABLE II
Comparison of Weight Gains of Male and Female Rats Continuously Fed Low Levels of Aflatoxin in Peanut Products With Those of Control Animals

Group No.[a]		Weight gain			
		52 weeks		104 weeks	
		M (g)	F (g)	M (g)	F (g)
A_0	Gen. I	369 ± 14(18)[b]	235 ± 9(17)	382 ± 32(6)	275 ± 16(4)
	Gen. IV	369 ± 9(30)	219 ± 5(23)	406 ± 16(11)	277 ± 18(4)
A_1	Gen. I	354 ± 7(19)	228 ± 4(19)	387 ± 8(16)	271 ± 10(12)
	Gen. IV	346 ± 5(29)	209 ± 3(25)	382 ± 7(23)	233 ± 8(19)
A_{10}	Gen. I	386 ± 14(16)	220 ± 7(19)	392 ± 30(5)	243 ± 23(4)
	Gen. IV	381 ± 10(25)	222 ± 4(27)	424 ± 16(12)	260 ± 17(10)
C_0	Gen. I	354 ± 12(12)	253 ± 7(23)	404 ± 14(3)	260 ± 13(19)
	Gen. IV	330 ± 22(14)	235 ± 10(14)	411 ± 15(6)	235 ± 22(6)[c]

[a] The subscripts indicate quantities of aflatoxin (ppb as B_1) as deliberate contaminants in the diets fed to these groups.
[b] The ± values are standard errors of the mean; the figures in parentheses are the numbers of rats in these experiments.
[c] Weight gain at 89 weeks.

TABLE III
Comparison of Longevity and Mortality of Male and Female Rats Continuously Fed Low Levels of Aflatoxin in Peanut Products With Those of Control Animals

Group No.[a]	Generation		Survival in weeks 75%	50%	25%	No. of animals surviving at termination of experiment/ no. of animals started
A_0	I	M	65	100	104	9/20
		F	81	92	104	7/20
	IV	M	92	104	104	16/30
		F	52	95	104	11/30
A_1	I	M	104	104	104	16/20
		F	89	104	104	12/20
	IV	M	104	104	104	24/30
		F	104	104	104	19/24
A_{10}	I	M	53	104	104	10/18
		F	82	102	104	8/19
	IV	M	70	104	104	17/24
		F	91	100	104	13/30
C_0	I	M	71	83	96	3/12
		F	75	96	100	8/23
	IV	M	37	83	88	5/19
		F	22	60	89	4/23

[a] Subscripts indicate quantities of aflatoxin (ppb B_1) as deliberate contaminants in the diets fed to these groups.

these were more frequently seen in the C_0 and A_0 groups, these changes were attributed to the aging process and to the strain of test rats used in the present study.

The liver pathology is reported in Table VI. The following lesions were found on histological review: parenchymatous nodules, bile duct cholangiomas and areas of retraction due to focal atrophy of the liver cords. On gross examination there was generalized mottling of the liver which appeared more frequently in the A_{10} group but which was also seen in the control groups. There were also depressed hemorrhagic areas. Although the abnormalities appeared in the control animals as well as in the aflatoxin-treated animals, there does appear to be an increase in the hepatotoxic effects with increased amounts of aflatoxin in the diet. However, the pathological findings were somewhat less extensive in the fourth generation animals indicating, perhaps, a tolerance developing for the toxin.

The animals fed the diet containing 80 ppb of aflatoxin (A_{80}) grew as well as the control animals for one year at which time half of the animals were killed (Table VII). In this experiment the liver

TABLE IV
Comparison of Reproduction and Lactation Performances of Rats Continuously Fed Low Levels of Aflatoxin in Peanut Products With Those of Control Animals

Group No.[a]	Generation	Successful pregnancies[b] (%)	Young alive at 21 days No.	Ave. wt. (g)
A_0	I	70 (14/20)	73	36.3
	II	80 (16/20)	81	39.9
	III	85 (17/20)	104	40.5
A_1	I	84 (16/19)	93	38.6
	II	80 (16/20)	68	37.5
	III	90 (18/20)	81	36.6
A_{10}	I	53 (10/19)	54	35.8
	II	85 (17/20)	85	39.3
	III	75 (15/20)	72	37.4
C_0	I	75 (15/20)	67	36.6
	II	80 (16/20)	77	40.3
	III	75 (15/20)	72	41.8

[a] Subscripts indicate quantities of aflatoxin (ppb as B_1) as deliberate contaminants in the diets fed to these groups.
[b] Females with litters surviving three days.

weight in both groups of animals was essentially the same, although liver lipid and liver cholesterol levels were higher in the animals fed the aflatoxin at 80 ppb than in the control group (Table VIII). Liver pathology is shown in Table IX. Here again no tumors were observed. The same type of lesions as had appeared in the first series of experimental animals were also seen here. At 52 weeks there was little difference between the control and aflatoxin-fed animals. However, after 76 weeks the incidence of lesions was considerably higher in the aflatoxin-fed animals than in the controls. But even after 90 weeks on the diets, no liver tumors were apparent in the few remaining animals.

Several investigators have reported the presence of well-defined hepatomas in rats fed aflatoxin at levels below those which were fed in our experiments (13,14). A comparison of the experimental conditions of Wogan and Newberne (14) with those used in these experiments revealed the following points of dissimilarity: (a) The strain of rats. Wogan and Newberne used Charles-River rats. In this experiment we used our own rat colony (USC strain). (b) The constituents of the diet. Wogan and Newberne used a purified diet containing 11% casein and 7.5% corn oil. Our semi-purified diet contained lactalbumin, skim milk, peanut protein and peanut oil. (c) The alflatoxin contaminant in the experiments of Wogan and Newberne was aflatoxin B_1 isolated from *Aspergillus flavus* (ATCG 15517) culture; it

TABLE V
Comparison of Weights, Lipid and Cholesterol Contents of Livers of Male and Female Rats Continuously Fed Low Levels of Aflatoxin in Peanut Products for Two Years With Those of Control Animals

Group No.[a]	Generation/ sex	Liver wt.	Liver (% wt. of total animal)	Total lipid (mg/g)	Total cholesterol (mg/g)
A_0	I M(9)[b]	9.2 ± 0.5[c]	2.1	44.9 ± 2.4	2.30 ± 0.10[c]
	F(7)	8.3 ± 0.5	2.8	40.7 ± 5.4	2.35 ± 0.30
	IV M(16)	9.7 ± 0.4	2.1	49.2 ± 2.8	2.88 ± 0.12
	F(11)	8.3 ± 0.2	2.7	33.5 ± 4.7	2.06 ± 0.12
A_1	I M(16)	9.8 ± 0.4	2.3	38.9 ± 2.3	2.07 ± 0.18
	F(12)	7.2 ± 0.3	2.4	40.9 ± 3.5	2.00 ± 0.09
	IV M(24)	10.4 ± 0.3	2.4	50.5 ± 3.7	2.81 ± 0.16
	F(19)	7.4 ± 0.3	2.6	40.9 ± 2.3	2.32 ± 0.16
A_{10}	I M(10)	10.9 ± 0.5	2.5	42.2 ± 5.0	2.45 ± 0.15
	F(8)	7.1 ± 0.3	2.5	42.2 ± 4.6	2.18 ± 0.18
	IV M(17)	10.3 ± 0.4	2.2	46.6 ± 2.2	2.79 ± 0.07
	F(13)	8.1 ± 0.4	2.7	40.0 ± 5.4	2.15 ± 0.05
C_0	I M(3)	11.6 ± 0.5	2.6	41.3 ± 5.3	2.07 ± 0.12
	F(8)	7.8 ± 0.5	2.6	42.0 ± 4.3	1.96 ± 0.06
	IV M(5)	12.2 ± 0.4	2.7	29.5 ± 1.4	2.03 ± 0.05
	F(4)	6.4 ± 0.6	2.3	38.1 ± 2.6	2.17 ± 0.09

[a] Subscripts indicate quantities of aflatoxin (ppb as B_1) as deliberate contaminants in the diets fed to these groups.
[b] Numbers in parentheses are numbers of animals on which determinations were made.
[c] The ± values in these columns are standard errors of the mean.

TABLE VI

Comparison of Liver Pathology of Male and Female Rats Continuously Fed Low Levels of Aflatoxin in Peanut Products With That of Control Animals

Group No.[a]	No. animals	Age wk.	Rats with lesions No. (%)	Parenchymatous nodules	Types of lesions (No.)		Focal hemorrhage or necrosis
					Cholangioma	Focal atrophy	
A_0	F 7	104	2(20)	2	0	1[b]	0
	M 9	104	1(11)	0	1	1[b]	0
A_1	F 11	104	10(91)	0	0	10	0
	M 16	104	6(38)	2	1	2	1
A_{10}	F 8	104	8(100)	5[b]	5[b]	2	0
	M 10	104	7(70)	5	2[b]	4	0
C_0	F 12	100	4(33)	0	1	1	2
	M 8	100	3(25)	1	0	2	0

[a] Subscripts indicate quantities of aflatoxin (ppb as B_1) as deliberate contaminants in the diets fed to these groups.
[b] More than one lesion per rat.

TABLE VII

Comparison of Weight Gains of Male and Female Rats Fed a High Level of Aflatoxin in a Peanut Product With Those of the Control Animals

Group No.[a]	Weight gain					
	26 weeks		52 weeks		76 weeks	
	M (g)	F (g)	M (g)	F (g)	M (g)	F (g)
A_0	359 ±9(23)[b]	235 ±5(23)	329 ±5(23)	209 ±5(23)	425 ±20(10)	234 ±6(9)
A_{80}	348 ±7(24)	223 ±3(23)	381 ±6(24)	222 ±3(23)	333 ±10(10)	217 ±8(9)

[a] The subscripts indicate quantities of aflatoxin (ppb as B_1) as deliberate contaminants in the diets fed to these groups.
[b] The ± values are standard errors of the mean; the figures in parentheses are the numbers of rats in these experiments.

TABLE VIII

Comparison of Weight, Lipid and Cholesterol Contents of Livers of Male and Female Rats Continuously Fed a High Level of Aflatoxin in a Peanut Product for One Year With Those of the Control Animals

Group No.[a]	Generation/ sex	Liver wt.	Liver (% wt. of total animal)	Total lipid (mg/g)	Total cholesterol (mg/g)
A_0	M(12)[b]	10.5 ± 0.2[c]	2.9	38.1 ± 1.6	1.76 ± 0.05
	F(12)	7.1 ± 0.3	3.0	34.2 ± 2.0	1.85 ± 0.03
A_{80}	M(12)	10.9 ± 0.4	2.7	49.0 ± 2.7	3.45 ± 0.20
	F(12)	6.3 ± 0.1	2.5	43.0 ± 3.1	2.82 ± 0.20

[a] Subscripts indicate quantities of aflatoxin (ppb as B_1) as deliberate contaminants in the diets fed to these groups.
[b] Numbers in parentheses are numbers of animals on which determinations were made.
[c] The ± values in these columns are standard errors of the mean.

TABLE IX

Comparison of Liver Pathology of Male and Female Rats Continuously Fed a High Level of Aflatoxin in a Peanut Product With That of Control Animals

Group No.[a]	No. of animals	Age wks.	Rats with lesions No./(%)	Parenchymatous nodules	Types of lesions (No.)		Focal hemorrhage or necrosis
					Cholangioma	Focal atrophy	
A_0	F 11	52	5(46)	1	0	2	2
	M 12	52	3(25)	1	0	0	3[b]
A_{80}	F 12	52	5(42)	1	0	1	3
	M 12	52	6(50)	1	0	1	4
A_0	F 4	76	1(25)	0	0	1	0
	M 4	76	1(50)	0	0	2	0
A_{80}	F 5	90	5(100)	3	0	5[b]	0
	M 5	90	4(80)	0	0	4	0

[a] The subscripts indicate quantities of aflatoxin (ppb as B_1) as deliberate contaminants in the diets fed to these groups.
[b] More than one lesion per rat.

TABLE X
Recent Observations on Critical Levels of Aflatoxin in Rations for Test Animals

Species	Aflatoxin B_1 in ration ppb	Test period months	Pathology assignable to aflatoxicosis	Investigator, year
Duckling	20	1	None	Melnick and Parker, 1963[a]
	50	1	Bile duct proliferation and nodule formation.	
Rat	10	24	None	Present study, Alfin-Slater et al., 1969
	80	21	Liver lesions noted but no hepatomas or other tumors.	
Swine	450(M)	8.3	None	Hintz et al., 1967 (15)
	450(F)	11	Minimal microscopic lesions in liver. No hepatomas or other tumors.	
Swine	400–600	3–6	Depressed growth; liver damage. No tumors.	Allcroft, 1965 (6)
Beef cattle	300	6.5	None	Garrett et al., 1968 (16)
	700	6.5	Increase in organ weights. Liver damage but no hepatomas or other tumors.	
Monkey	70	36	None	Cuthbertson et al., 1967 (17)
	360	36	None	
	1800	36	Some liver pathology in survivors but no hepatomas or other tumors.	

[a] Unpublished studies based upon 30 day feedings to ducklings of a ration containing the natural aflatoxin contaminants in peanut discards.

was then dissolved in acetone and added to the casein. The aflatoxin used in our experiments was derived from a mixed strain which was a natural contaminant of peanuts. Since the aflatoxin was fed as ground, roasted peanuts, this aflatoxin had gone through the heating conditions associated with the roasting of peanuts to make them palatable for human consumption.

We are now engaged in a study where we are feeding our diet to Charles River rats and the Wogan-Newberne diet to our rats to see whether it is the use of our diet or our strain of rats which is responsible for the absence of hepatoma formation in our studies.

There is no doubt that the high aflatoxin-containing diet produces a toxic effect on liver as is manifested by the formation of lesions. However, the relationship of these lesions to hepatoma formation has not been elucidated. If the lesions are related to a precancerous state, then at some concentration and at some time period tumors should develop in the liver of our rats. Studies have been appearing recently in which other investigators have failed to find tumors in experimental animals given aflatoxin (15–17). In one investigation (18), liver biopsies were performed on two young children who had unintentionally eaten large quantities (70–140 g per day) of aflatoxin-contaminated (up to 1000 ppb) cereal over a 10 month period beginning when the children were under one year of age. Observations made on these children, four and six years after they had subsisted on this diet, revealed no malignant hepatomas; there was, however, a hepatic fibrosis.

The diet in the present study provided about 50% of the calories from the ground roasted peanut component. If we assume that the average intake of a peanut product per day per individual is somewhat less than 1 oz, or about 5% of the caloric intake, and if results on rats are translatable to humans, our studies indicate that roasted peanuts containing 30 ppb or less of aflatoxin provided a significant margin of safety, and that as a result of in-plant processing where objectionable peanuts are removed and discarded as unfit for human consumption, the margin of safety becomes more than 100-fold.

In conclusion, it appears that there may be a tolerance for aflatoxin. This is certainly true in the case of one strain of rat, the former USC strain, under the conditions of test employed in the present study. Of particular importance is the finding that the borderline level of toxicity of aflatoxin is 10 ppb of the diet as aflatoxin B_1 or somewhat greater. This is in a diet providing 35% by weight of ground roasted peanuts in the form of a simulated peanut butter. Hence, the borderline level for the peanut moiety of the diet (sole source of aflatoxin) is at least 30 ppb. To attain this very high level of aflatoxin in the ground roasted peanuts required a 10 fold return of the roasted pick-outs that are normally discarded in regular processing. Even at the extraordinarily high concentration of 80 ppb of aflatoxin B_1 in the diet, equivalent to approximately 240 ppb in the ground roasted peanut component, no tumors of any type were observed with the test animals even after prolonged feedings. In a recent report (19), swine raised from weanling to 200 lb body weight on rations containing varying levels of aflatoxin showed no effects at a level of 233 ppb aflatoxin and no evidence of aflatoxin residues in meat, blood or liver. Similarly, beef steer suffered no ill effects from 300 ppb aflatoxin fed for five months. As more studies are reported in this field (Table X) it appears that high levels of aflatoxin consumption are required for tumor development. With all species studied, the duck, rat, swine, beef cattle and the monkey, the dietary level for tumor formation is well above 30 ppb of aflatoxin B_1.

REFERENCES

1. Blount, W. P., J. Brit. Turkey Fed. *52*, 55–58 (1961).
2. Carnaghan, R. B. A., and K. Sargeant, Vet. Record *73*, 726–727 (1961).
3. Allcroft, R., R. B. A. Carnaghan, K. Sargeant and J. O'Kelly, Ibid. *73*, 428–429 (1961).
4. Sargeant, K., A. Sheridan, J. O'Kelly and R. B. A. Carnaghan, Nature (London) *192*, 1096–1097 (1961).
5. Carnaghan, R. B. A., R. D. Hartley and J. O'Kelly, Ibid. *200*, 1101 (1963).
6. Allcroft, R. in "Mycotoxins in Foodstuffs," Edited by G. N. Wogan, M.I.T. Press, Cambridge, 1965, p. 153.
7. Lancaster, M. C., F. P. Jenkins and J. McL. Philp, Nature (London) *192*, 1095–1096 (1961).
8. Butler, W. H., and J. M. Barnes, Brit. J. Cancer *17*, 699–710 (1964).
9. Barnes, J. M., and W. H. Butler, Nature (London) *202*, 1016 (1964).
10. Salmon, W. D., and P. M. Newberne, Cancer Res. *23*, 571–576 (1963).
11. Milner, M. in "Mycotoxins in Foodstuffs," Edited by G. N. Wogan, M.I.T. Press, Cambridge 1965, p. 69.
12. J. Assoc. Offic. Agr. Chemists, "Changes in Methods: 25. Nuts and Nut Products," *49*, 229–231 (1966).
13. Butler, W. H., and J. M. Barnes, Fd. Cosmet. Toxicol. *6*, 135–144 (1968).
14. Wogan, G. N., and P. M. Newberne, Cancer Res. *27*, 2370–2376 (1967).
15. Hintz, H. F., H. Heitman, Jr., A. N. Booth and W. E. Gagné, Proc. Soc. Exptl. Biol. Med. *126*, 146–148 (1967).
16. Garrett, W. N., H. Heitman, Jr. and A. N. Booth, Ibid. *127*, 188–190 (1968).
17. Cuthbertson, W. F. J., A. C. Laursen and D. A. H. Pratt, Brit. J. Nutr. *21*, 893–908 (1967).
18. Payet, M., J. Cros, C. Quenum, M. Sankale and M. Moulanier, Presse Med. *74*, 649–651 (1966).
19. Booth, A. N., Abstract No. 154, AOCS Meeting, San Francisco, 1969.

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