EFFECT OF VARIOUS TEMPERATURES ON THE PRODUCTION OF AFLATOXINS IN FOODS

by

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INTRODUCTION

Since the initial discovery that certain strains of the mold Aspergillus flavus produce aflatoxin (Blount, 1961), numerous studies have been initiated concerning the role this organism might play in our food chain relationship (Kraybill, 1969).

Due to the lack of human pharmacological data, it has not been possible to establish tolerances for this common environmental contaminant; however, toxicological and clinical findings in nonhuman primates have been extrapolated to man. Cuthbertson et al. (1967) found the no-effect level for aflatoxin B_1 in monkeys was 0.3 p.p.m. in the diet or equivalent to a daily consumption of 0.015 mg. of aflatoxin/kg. of body weight. Subacute toxic effects in the primate have been studied by Tulpule et al. (1964). Monkeys fed either 0.5 or 1.0 mg. of aflatoxin B_1 for 18 days and 1.0 mg. daily thereafter had primary lesions of portal inflammation and fatty change in their livers.

Pregnant animals are highly susceptible to aflatoxin. Fetal development has been retarded when the mother was stressed with aflatoxin B_1 (Le Breton et al., 1964). Elis and Di Paola (1967) noted teratogenic effects due to aflatoxin. It is possible that long term ingestion of foods contaminated with aflatoxin may have adverse effects upon the human fetus. This lends further support to the statement by Kraybill (1969) that any demonstrable level of aflatoxin should be considered proof of contamination.

In an effort to determine the ability of A. flavus to produce aflatoxin at household refrigerator temperatures, Van Walbeek et al. (1969) inoculated strains of the toxin-producing mold on solid and liquid YES media and incubated them at 7.5 and 10 C. All cultures produced significant

amounts of aflatoxin.

The present study was initiated to determine if A. flavus would produce aflatoxin in foods incubated in household refrigerators. Cheddar cheese and luncheon meat was inoculated with an aflatoxin-producing strain of A. flavus. Each sample was wrapped in tin foil and incubated for 12 days at either 4.4 or 7.2 C. Samples were then evaluated for aflatoxin production.

MATERIALS AND METHODS

A. flavus Cultures

Cultures of A. flavus, ATCC 15517[1], were utilized for inoculation of samples. The method described by Davis et al. (1966) was employed for culture production. Cultures were incubated at 25 C for 2 weeks and placed in an ultra-low temperature freezer[2] at 4 C until used.

To determine viability of the culture and to quantitate aflatoxin production, a slant was removed from the freezer and allowed to thaw to room temperature. Ten ml. sterile YES medium (2% yeast extract plus 20% sucrose in distilled water) was added to the slant. A spore suspension was made by gently agitating the growth with a sterile loop. An inoculum of the spores was transferred to a culture slant of Sabouraud dextrose[3] agar with a 1/100 sterile graduated wire loop. The slant was incubated at room temperature for 8 days, at which time aflatoxin production was expected to

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[1]American Type Culture Collection, Rockville, Maryland.

[2]Revco, Inc., Deerfield, Michigan.

[3]Difco Laboratories, Deerfield, Michigan.

be maximal. An aflatoxin estimation of this slant was found to be quantitatively greater than a 10 ul. standard containing 1.0 ug. B_1/ml and 1.0 ug. G_1/ml.

Aflatoxin Assays of Meat and Cheese Samples

Extraction. Extraction was accomplished with the method described by Pons et al. (1968). After being reduced to dryness and adding 0.5 ml. of chloroform, the vials were tightly sealed and stored at 4.4 C until quantitative assays could be performed by (TLC).

Preparation of TLC plates. Precoated TLC Silica Gel sheets on aluminum[4] were used for quantitative assay. The sheets were placed in a 121.1 C oven for 30 minutes to ensure dryness prior to spotting the samples.

Thin layer chromatography. Five and 10 ul. sample portions were spotted 2 cm. from the bottom of the sheet using a calibrated automatic pipette[5]. On the same sheet a 10 ul. portion made from a 20 ul. combination of sample and standard was spotted. Five and 10 ul. portions of the standard were also spotted on the same sheet. Plates were developed in a chromogram chamber[6] with a trough containing 50 ml. of a 1/9 acetone and chloroform, V/V, as the developer. The plates were developed at room temperature until the solvent front reached 15 cm. above the initial spotting points. The sheets were allowed to air dry and illuminated by placing the sheets flat,

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[4]E. Merck A G Darmstadt, Germany, distributed by Brinkman Instruments, Inc., Westbury, New York.

[5]Oxford Sampler, Oxford Laboratories, San Mateo, California.

[6]Distillation Products, Inc., Rochester, New York.

coated side up on a long wave ultraviolet light[7] in a dark room.

Aflatoxin quantitation was made by visual comparison of the intensity of fluorescence of the unknown to that of the standard, using the formula micrograms/ml. = (SxYxV)/(XxW), where S = ul. aflatoxin standard equal to corresponding sample spot; Y = concentration of specific aflatoxin in standard solution in mg./ml.; V = volume in ul. to which sample extract is finally diluted for TLC; X = ul. sample extract spotted giving fluorescence equal to aflatoxin standard; W = grams of sample originally used times 0.6.

The quantitative standard, SA 5-7[8], was prepared from pure crystalline aflatoxins. The sealed ampoule contained 5 ml. of a stock chloroform solution with 5.0 ug. of B_1; 1.5 ug. of B_2; 5.0 ug. of G_1 and 1.5 ug. of G_2/ml. When diluted for analytical purposes the standard contained B_1-.0001 ug.; B_2-.0003 ug.; G_1-.0001 ug. and G_2-.0003 ug./ul.

The diluted standard was contained in a tightly sealed vial, wrapped in aluminum foil and stored at 4.4 C until TLC spotting.

Equipment preparation. In order to simulate household conditions two refrigerators[9] were provided with samples for a 12 day period. One refrigerator was set at 4.4 C and the other at 7.2 C. A maximum-minimum thermometer[10] was placed in each refrigerator and monitored for 2 weeks prior to inoculating the samples. It was determined that each refrigerator

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[7]Mineralight Model UVSL-25, Ultraviolet Products, Inc., San Gabriel, California.

[8]Supplied by Dr. Leo A. Goldblatt, A.R.S., USDA, New Orleans, Louisiana.

[9]Frigidaire, Div. of General Motors, Detroit, Michigan.

[10]Taylor Instrument Companies of Canada Limited, Toronto, Canada.

accurately maintained the specified temperature during the study.

Experimental Samples and Procedures

Cheddar cheese and variety packs of luncheon meat containing salami, New England loaf and bologna were selected as samples for this project. The cheese was purchased in one pound blocks from a local cheese factory. The luncheon meat was bought from the shelf of a local grocery store.

In order to simulate normal opening and closing of the refrigerator during daily use, each refrigerator was opened three times daily (morning, noon and evening) and left open for 3 minutes. This procedure was performed each day throughout the 12-day incubation period. Each refrigerator temperature increased an average of 8 degrees prior to closing the door. Both refrigerators regained their holding temperatures 15 to 20 minutes after the doors were closed.

A total of 58 cheese samples were sliced into blocks weighing between 16 and 22 gm. Also, 58 meat samples were sliced into portions weighing between 4 and 7 gm.

A suspension of A. flavus spores were made as previously described. A total of 42 cheese and 42 meat samples were inoculated on their top surfaces in an enclosed hood[11] in order to inhibit contamination. A single swath was made on the surface of each sample with a 1/100 graduated wire loop. A total of 16 cheese and 16 meat samples were not inoculated and served as controls. Each sample was loosely wrapped in aluminum foil that had been previously identified.

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[11] Isolator/Lab., Fisher Scientific Co., Pittsburgh, Pennsylvania.

The inoculated samples were handled in the following manner: cheese and meat samples were divided into four groups, the first three containing 12 meat and 12 cheese samples per group and the fourth contained six cheese and six meat samples. The first three groups were further divided into two subgroups each containing six cheese and six meat samples. In group one each subgroup was inoculated and immediately placed into either a 4.4 or 7.2 C refrigerator. Group two was inoculated and held at 25 C for 2 hours prior to refrigeration and group three was held for four hours before refrigerating. The fourth group was inoculated and held at 25 C without refrigeration for the 12 day incubation period. Control samples were divided into four groups each consisting of four cheese and four meat samples. The first three groups were further divided into subgroups consisting of two cheese and two meat samples. The fourth group was not divided into subgroups. Each group and its subgrouping were handled in the same manner as previously described except they were not inoculated (Table 1). All samples were incubated for 12 days and then placed in a freezer at 4 C until they could be assayed.

RESULTS

Aflatoxin Assays of Samples

The samples which had visible mycelial growth prior to assay and which were positive for aflatoxin production on TLC sheets contained two aflatoxins, B_1 and G_1. The G_1 aflatoxin was also present in very small amounts and was not recorded in the data book.

Aflatoxin assays were determined on cheese and meat samples that were

TABLE 1.--Incubation procedure for cheese and meat samples.

Inoculated Samples							
12C[+] 12M[*]		12C 12M		12C 12M		6C 6M	
		Held 2 hrs. at 25 C		Held 4 hrs. at 25 C		Held at 25 C for 12 days Not Refrigerated	
6C-6M	6C-6M	6C-6M	6C-6M	6C-6M	6C-6M		
4.4 C	7.2 C	4.4 C	7.2 C	4.4 C	7.2 C		
Control Samples							
4C 4M		4C 4M		4C 4M		4C 4M	
		Held 2 hrs. at 25 C		Held 4 hrs. at 25 C		Held at 25 C for 12 days Not Refrigerated	
2C-2M	2C-2M	2C-2M	2C-2M	2C-2M	2C-2M		
4.4 C	7.2 C	4.4 C	7.2 C	4.4 C	7.2 C		

[+]Cheese samples. [*]Meat samples.

inoculated and left at 25 C for the 12 day incubation period first. Table 2 shows the results of quantitative assays determined on the six cheese samples. Each sample had visible mycelial growth on the surface of the cheese prior to assay determination. Each was found to be positive for B_1 aflatoxin ranging from 10.8-13.7 p.p.b. with an average of 11.9 p.p.b. Table 3 shows the results of the luncheon meat inoculated and placed at 25 C for 12 days. Visible mycelial growth was slight on the surface of each sample. B_1 aflatoxin was present in each sample, however two samples showed only a slight fluorescence and they were recorded as traces. The remaining four samples contained aflatoxin in amounts varying from 7.14-10.00 p.p.b. with an average of 8.45 p.p.b.

Quantitative assays were performed on the cheese and meat samples that were inoculated and refrigerated immediately following inoculation or held at 25 C for 2 or 4 hours prior to refrigeration. All of these samples were found to be negative for mycelial growth or the presence of aflatoxin. Likewise, the 32 meat and cheese samples not inoculated and serving as controls were found to be negative for mycelial growth or aflatoxin presence.

After 15 samples had been run through the column chromatography process, the column contents were removed and replaced with fresh materials to be sure that no fluorescing spots or interferring compounds would be present on TLC.

DISCUSSION

The results show that A. flavus grew well at 25 C which closely correlates with previous findings of an optimal growth range of 30-40 C (Raymond, 1966).

TABLE 2.--Aflatoxin content of cheese samples inoculated and not refrigerated.

Sample #	1	2	3	4	5	6
Wt. in gm.	22	18	23	22	20	21
B_1 content in p.p.b.	11.3	13.7	10.8	11.3	12.5	11.9

TABLE 3.--Aflatoxin content of meat samples inoculated and not refrigerated.

Sample #	1	2	3	4	5	6
Wt. in gm.	1	7	6	7	5	6
B_1 content in p.p.b.	tr.*	7.14	8.33	tr.*	10.0	8.33

*Recorded as a trace.

Yields of aflatoxin recovered in this study are less than those obtained by Van Walbeek et al. (1969), however their use of malt yeast agar and broth as the medium probably enhanced the production of greater amounts of toxic metabolites.

Because of the lack of pharmacological data on man it was not possible to determine whether the amounts of aflatoxin recovered were sufficient to cause aflatoxicosis in man. However, Van Walbeek et al. (1968) reported an illness in a child from a sample of spaghetti containing 12.5 p.p.b. aflatoxin. Leistner and Ayres (1966) conducted a study to determine whether potentially toxinogenic mold species are commonly associated with country cured hams and fermented sausages. Molds of the genus Aspergillus were recovered from 90% of the hams and 33% of the sausages. Because of the highly toxinogenic capabilities of certain strains of Aspergillus it seems that the hazards far outweigh the beneficial influence that molds are thought to contribute to cured meats. Further work in this area is certainly warranted in the interest of public health.

In this study samples refrigerated immediately following inoculation as well as those pre-incubated at 25 C for 2 and 4 hours prior to refrigeration failed to produce aflatoxin. It can therefore be assumed that even though food might become contaminated and left at 25 C for periods of up to 4 hours there appears to be no danger of aflatoxin production if the food is refrigerated at a temperature of 4-8 C.

CONCLUSIONS

A. flavus will grow and produce aflatoxin on cheddar cheese and luncheon meat at 25 C.

Refrigeration of perishable foods appears to be an effective preventive measure towards preventing growth and production of aflatoxin by A. flavus even though the food may have been contaminated with the mold.

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REVIEW OF LITERATURE

The Discovery of Aflatoxin

In 1960, over 100,000 turkeys died from an outbreak first called "Turkey X" disease by Blount (1961) in England. Not long afterwards cases were reported in calves (Allcroft et al., 1961) and swine (Harding et al., 1963).

The common factor in all the outbreaks was found to be peanut meal imported from Brazil. Collaborative work between the Tropical Products Institute and the Central Veterinary Laboratory at Weybridge, England, eventually obtained the toxic product in crystalline form and determined it to be highly fluorescent under ultraviolet light as well as highly fatal in susceptible species when fed the extract in their ration (Allcroft et al., 1961).

Identification of the toxic substance as a metabolite of a mold was made by Sargeant et al. (1966).

Aflatoxins display acute toxicity to several species of animals and likewise have been shown to possess carcinogenic properties in certain animal species as well. Discovery of aflatoxins in certain agricultural products and demonstrations of certain biological effects to animals fed contaminated rations have emphasized the potential public health hazard which could arise from contamination of our food supplies with this fungal toxin (Kraybill and Shimkin, 1964).

Since the original isolation of aflatoxin from peanut meal investigators have also isolated it from the following products; barley, brazil nuts, cocoa beans, corn, cottonseed, raisins, soya bean meal and wheat (Goldblatt, 1966).

Physical and Chemical Properties

The aflatoxins are closely related and highly substituted compounds. The presence of furocoumarin configuration places them among a large group of naturally occurring compounds with many pharmacological activities.

Because of the close similarities in structural configuration, the infrared absorption spectra of the four compounds are also quite similar. The fluorescence emission maximum for B_1 and B_2 has been reported as 425 mu and for G_1 and G_2 as 450 mu (Hartley et al., 1963).

Although aflatoxins are rather stable compounds they will undergo degradation under several conditions. They will partially decompose upon standing in methanolic solution and the process is greatly accelerated in the presence of light. Degradation also occurs on chromatograms exposed to air and ultraviolet light or visible light (Wogan, 1966). They are able to survive autoclaving (Hesseltine et al., 1966) and are resistant to several chemical agents (Trager and Stoloff, 1967).

Aflatoxin Producing Fungi

The toxins were designated as aflatoxins because they were produced by the common mold Aspergillus flavus. At least four other species of the genus Aspergillus (A. niger, A. parasiticus, A. ruber and A. wenti) have been reported to produce aflatoxin (Kulik and Holaday, 1966).

The identical compounds may also be produced by a completely different genus of molds, the Penicillium. The following all have been reported to produce aflatoxins; P. citrinum, P. frequentans, P. puberulum and P. variable (Hodges et al., 1964; Kulik and Holladay, 1966). However, Wilson et al. (1967) reported they failed to confirm the aflatoxigenecity of 8 P. puberulum cultures including the isolate described by Hodges et al.

(1964). Also, reservations have been expressed as to the production of aflatoxins by other species of Penicillium.

Characteristics of Aspergillus flavus

Some 75% of all A. flavus-oryzae strains examined at the Tropical Products Institute in England have been found to be toxin producers.

Optimum growth temperature is in the 30-40 C range. The optimum moisture content has been found to be in the range of 15-28%. They have not been found to grow on material having a moisture content of less than 8%. Also, they have not been found to grow well outside the 12-47 C temperature range (Raymond, 1966).

Crops may become contaminated with A. flavus in the field but it is generally agreed to be a storage mold of crops. Peanuts are the greatest aflatoxin hazard if they are insufficiently dried or if they are improperly stored.

Morphlogically, A. flavus differs from other species of the genus in that the conidial heads are globose to columnar and light yellow-green to olive-brown; conidiophores are usually colorless and roughened; conidia are round and usually roughened; and vesicles are fertile over most of their surface. On Czapek's agar the growth is tight velvety to coarse in texture and yellow-green to jade or olive-green (Raper and Fennel, 1965).

Extraction of Aflatoxin

In original investigations it was demonstrated that the compounds could be extracted with methanol (Allcroft et al., 1961). Since the original work a variety of procedures have been developed particularly for assays of agricultural products for presence of aflatoxin. The extracts produced by

these procedures are separated into their components by chromatographic techniques. Thin layer chromatography is utilized for separation on silica gel plates developed with 3 to 5% methanol in chloroform (Nesheim, 1964).

Chromatograms of extracts containing aflatoxins viewed under ultraviolet light emit fluorescent compounds. Aflatoxins B_1 and B_2 emit blue visible light, G_1 and G_2 emit yellow-green visible light. The amount and relative proportion of those four compounds present in extracts are variable. Such factors as mold strain, the medium composition and culture conditions are all variable factors. B_2 and G_2 are normally present in small amounts whereas B_1 is usually present in highest amounts (Wogan, 1966). These four compounds were all isolated by workers in England (Nesbitt et al., 1962).

Biological Effects of Aflatoxin

Acute and subacute toxicity in vivo. The discovery of aflatoxins as contaminants of feeds and the potential public health hazard has stimulated a considerable amount of research in various biological assay systems. Animals fed sufficiently large acute doses have been found to succumb easily. Subacute doses have been found to cause histological changes and chronic exposure for extended periods has resulted in tumor induction in several animal species.

Early studies have suggested that the duckling is the species most susceptible to acute poisoning. However, studies by Wogan (1966) have indicated that the dog, rabbit and guinea pig have LD_{50} values in the same order of magnitude as the duckling.

LD_{50} values have been worked out for several species of animals; duckling, rat, hamster, guinea pig, rabbit and dog. They are calculated from mortality over 7 day periods. In most species death occurs in 72 hours

after administration of the compound and necropsy reveals gross liver damage. In all species studied sensitivity decreases with age.

The relative lethal potencies of the four aflatoxins have been calculated by Carnaghan et al. (1963) for the day old duckling. Oral 7 day LD_{50} values for each compound are: aflatoxin B_1, 18.2 ug; B_2, 84.8 ug; G_1, 39.2 ug; and G_2, 172.5 ug on a 50 gm. weight basis. Aflatoxin B_1 is the most potent followed by G_1, B_2 and G_2 in order of decreasing potency.

Subacute toxicity. Few subacute toxicity studies have been conducted for various animal species. However, field cases of various animals which consumed sub lethal quantities of aflatoxins for several days or weeks commonly exhibit moderate to severe liver damage as the predominant pathological sign. From observations from these field cases two conclusions have been made and verified. First, biliary hyperplasia is the most consistently observed lesion which occurs in all species except the sheep. Also, the sheep is resistant to the effects of aflatoxin. It has been concluded that sheep excrete aflatoxin through gut rather than transport this toxin through the liver for detoxification (Allcroft, 1965).

In the primate subacute toxic effects have been studied by Tulpule et al. (1964). Young Rhesus monkeys were fed either 0.5 or 1.0 mg. of aflatoxin for 18 days, then 1.0 mg. from then on. Histopathology studies of these livers showed portal inflammation and fatty change to be the primary lesion.

Sublethal toxicity. Prolonged administration of sublethal quantities of aflatoxin contaminated food will cause yet another effect. Investigators at the Unilever Research Laboratories in England fed diets containing highly toxic peanut meals to rats. After 6 months nine of 11 rats

developed multiple liver tumors. This was the first report of the carcinogenic properties of the aflatoxins. Since this initial discovery much work has been performed to determine precise dose-response conditions. Newberne (1965) has reported good correlation between liver tumor incidence and dietary aflatoxin content with a range of .06 to 1.8 p.p.m. of aflatoxin. A 90% tumor incidence in rats occurred when fed the highest level for 370 days.

Studies by Barnes and Butler (1964) indicate that continuous feeding is not required for hepatome induction in rats. All rats fed a ration containing 80% aflatoxins developed liver cancer 300 days following withdrawal. Since information is lacking on an induction period for tumor production in either man or monkey, no conclusions can be reached as to chronicity of aflatoxin hazard in an epidemiological situation.

O'Gara (unpublished) reports that 18 monkeys from a group of 62 died in a 6 to 24 month study due to aflatoxin administration. The newborn and infants were found to be more susceptible than older animals. The maximum tolerated dose was 2500 ug/kg. I.P. given weekly and 400 ug/kg. per os given at the rate of three to five doses per week. This age relationship is borne out in other animal species.

This data fortifies the potential acute hazard of aflatoxin to man. It can be generally stated that the toxicity of the aflatoxins varies greatly depending upon the species tested, age, sex and nutritional status of the species. The dose applied and length of exposure are also variant factors in the degree of toxicity shown.

Specific Actions of Aflatoxins

Being comparable to other mycotoxins, aflatoxins express a toxic

response in the liver as the primary target organ.

Utilizing heteroploid human embyonic lung cells, Legator et al. (1965) found that aflatoxin introduced into these cell cultures suppressed mitosis and DNA synthesis, leading to an increase in giant cell formation. Childs and Legator (1966) report that treatment of the cells results in a 15-30% inhibition of DNA synthesis in 24 hours and a 75-80% inhibition in 48 hours. Protein synthesis unaffected at 24 hours is reduced 40% at 48 hours and 85% at 72 hours. In contrast to the decrease in DNA and protein synthesis the specific activity of thymidine kinase continues to increase from 24 to 72 hours in culture. Thymidine kinase activity has been correlated with cell replication.

In addition to the above effects it has been found that pregnant animals have a high degree of susceptibility to aflatoxin. The fetus has been observed to be retarded when the animal was stressed with B_1 aflatoxin (Le Breton et al., 1964). Elis and DiPaolo (1967) noted teratogenic effects of aflatoxin. It is possible, with respect to human implications, that subchronic to chronic ingestion of foods contaminated with aflatoxin may have adverse effects upon the fetus.

Food Chain Relationship

Although aflatoxin has been detected in peanut meals fed to cows and pullets, it has not been detected in an egg or cow's liver (Allcroft and Carnaghan, 1962). Other reports substantiate the fact that animal tissues may not contain any aflatoxin. However, since the discovery of the liberation of aflatoxin in the milk of cows fed aflatoxin in the ration an epidemiological problem of great magnitude could be in the making. Although Allcroft and Carnaghan (1962) found no detectable toxin in British milk

supplies the probability of contamination and exposure to the aflatoxin via the family cow should be considered epidemiological since there is no opportunity for dilution by bulking or pooling of milk.

Since the initial discovery of aflatoxin in milk it is now known that there are really two new toxic materials in the milk. During 1966 they were christened aflatoxin M_1 and M_2, since they were first found in milk (Allcroft et al., 1966).

The two other and most recently discovered aflatoxins were isolated late in 1966 from cultures of Aspergillus flavus and were designated as aflatoxin B_{2a} and aflatoxin G_{2a} (Dutton and Heathcote, 1966).

Only recently have mycotoxicoses been recognized as a potentially serious public health problem. Alarm has been widely expressed at the amount of aflatoxin in peanuts and other protein supplements. In August 1966 the Protein Advisory Group settled upon a maximum level of .03 mg/kg. (30 p.p.b.) of foodstuffs (Anonymous, 1966). The group would have preferred to impose a lower level in order to provide a wider margin of safety but felt that there was an even more urgent need to provide extra protein in some parts of the world in order to prevent malnutrition.

Due to the lack of pharmacological data on man, toxicological and clinical findings on nonhuman primates have been utilized to extrapolate to human safety. Cuthbertson et al. (1967) found a no effect level for aflatoxin in monkeys was 0.3 p.p.m. in the diet or equivalent to a daily consumption of 0.015 mg. of aflatoxin per kg. of body weight. It is customary to apply a safety factor of 50 for the minimal safe dose and assuming equivalence in response to man this represents 0.3 ug of aflatoxin per kg. of body weight. However, Kraybill (1969) states that these calculations

include several built-in deductions which may not hold, namely, (1) that the liver response in rats may have relevance to nonhuman or human primates, (2) that the short term feeding results in monkeys are comparable to those of the rat, and (3) that man will show a response similar to that of other species investigated.

Because of the lack of definitive pharmacological data of aflatoxin in man, it has not been possible to establish tolerances for this naturally occurring environmental contaminant. Any demonstrable level of aflatoxin should be considered as proof of excessive contamination with fungal metabolite toxins. Therefore, if such contamination can be proved, either the product must be rejected or remedial measures should be taken to control the fungal toxin (Kraybill, 1969).

Investigations of Aflatoxin in Human Food

Leistner and Ayres (1966) conducted a study to determine the beneficial effects molds might have on country cured hams and fermented sausages. Also added to the study was whether potentially toxinogenic mold species are commonly associated with meats. Molds were isolated from the surface of all hams and sausages examined. Molds of the genus Aspergillus were recovered from 90% of the hams and 33% of the sausages. Heavy mold growth commonly occurs on the surface of country cured hams and is regarded as an indication of good quality. Because of the highly toxinogenic capabilities of certain strains of Aspergillus it seems that the hazards far outweigh the beneficial influence it is often thought that molds contribute to cured meats.

Bullerman et al. (1969) recently reported aflatoxin production on fresh beef, ham and bacon inoculated with toxic strains of A. flavus and A.

parasiticus. Samples were stored at 15, 20 and 30 C. Meats stored at 10 C were spoiled by bacteria and yeast before detectable levels of aflatoxin were produced. High levels of aflatoxin were produced in meats stored at 20 C, one sample supported the production of 630 ug of aflatoxin per gram of meat. Meats stored below 30 C developed higher levels of G_1 than B_1, but at 30 C A. flavus produced equal amounts of B_1 and G_1.

Recent work by Van Walbeek et al. (1968) indicates that our food supplies may be becoming highly contaminated with fungi that produce aflatoxin. It was found that three food samples associated with illness harbored fungi that produce aflatoxins. A sample of dry spaghetti, in which small amounts of aflatoxin were found (12.5 p.p.b.) was implicated in the illness of one child who required hospital treatment for a few days. It was also found that over one-half of the samples of food from consumers complaining about moldiness were contaminated with aflatoxin producing aspergilli. It would seem that the time has arrived when we should be routinely checking for toxinogenic strains of molds as well as bacterial contamination when investigating food borne illnesses.

The work of Van Walbeek et al. (1969) questions the previously held concept that proper refrigeration of foods prevents aflatoxin production even though the food may be contaminated with Aspergillus flavus. Strains of A. flavus that produce high concentrations of aflatoxins at room temperature also produced these toxins in significant concentrations under conditions simulating household refrigeration (7.5-10 C). The rate of toxin production was markedly influenced by preincubating cultures at room temperature for 24 hours. The possibility must therefore be considered that toxin may be formed in foods stored in wrappers or containers that would create conditions comparable to those found in small culture vials.

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EFFECT OF VARIOUS TEMPERATURES ON THE PRODUCTION OF AFLATOXINS IN FOODS

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Information is lacking in the literature regarding the ability of the common mold contaminant Aspergillus flavus to grow and produce aflatoxin in perishable foods at normal refrigeration temperatures. Because of its public health importance the possibility of perishable food becoming contaminated with the mold and the effect of refrigeration on production of aflatoxin was investigated.

Cheddar cheese and luncheon meat samples were inoculated with Aspergillus flavus ATCC 15517. The samples were placed in either a 4.4 or 7.2 C refrigerator immediately, or were pre-incubated at 25 C for 2 or 4 hours prior to refrigeration to simulate transportation time from store to household. Other samples were kept at 25 C for a 12 day period. Uninoculated cheese and meat samples served as controls and were handled in the same manner. Samples were quantitatively analyzed for aflatoxin production by thin layer chromatography.

All samples except those inoculated and incubated at 25 C were found to be negative for aflatoxin production. The results of this study indicate that the mold will not produce aflatoxin in the tested foods if they are kept at normal refrigeration temperatures.