AFLATOXINS AND OTHER MYCOTOXINS IN AGRICULTURAL PRODUCTS

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Molds of many types have long been recognized as spoilage agents of many different foods. Growth of molds invariably has been associated with the formation of surface colonies and consequent discoloration of the food. Flavor defects and changes in the physical nature of the product often accompany development of the mold. When molds appear on certain foods such as cheese, the obviously molded area is often removed, and the remainder, unless it is deteriorated, is considered satisfactory for use as a food. Although this practice has been common for years, its safety must be re-evaluated in the light of what has recently been learned about mycotoxins. Mycotoxins are toxic metabolites produced by certain molds during growth on a suitable substrate. There are undoubtedly many unreported instances in which moldy feed or food caused illness in animals or humans. Two of the most dramatic to be reported occurred in Russia and England.

Wartime conditions during the Falls of 1942, 1943, and 1944 resulted in incomplete harvesting of cereal grains grown in the Orenburg district and in some other areas of Russia. After overwintering in the field, grains were harvested the following spring and were used to prepare foodstuffs. Consumption of these food products resulted in frequent and fatal outbreaks of a condition designated as septic angina or toxic alimentary aleukia (15). Production of the toxic substance(s) was associated with the development of certain fungi on the grain while it was covered with snow. Organisms principally responsible for this mycotoxicosis were found to be in the genera Fusarium and Cladosporium (14).

Eighteen years later, early in 1960, outbreaks of what seemed to be a new disease caused heavy losses among young turkey poults on a number of farms in southern England. It has been estimated that at least 100,000 poults died during this outbreak. The disease was characterized by depression, a staggering gait, and sudden death. The turkey carcass was usually congested and edematous and the liver was enlarged, pale, and firm. Later outbreaks of a similar nature were reported in ducklings and young pheasants. One farmer alone is believed to have lost about 10,000 ducklings. Ducklings seemed to be very susceptible to the toxic substance, and in addition to liver lesions, many had extensive subcutaneous hemorrhages of the legs, feet, and back. The source of toxic material was found to be peanut meal used in the diet and imported from Brazil. Cultural examination of the peanut meal resulted in the isolation of a strain of Aspergillus flavus possessing the ability to produce the toxin present in the peanut meal (10).

These two examples are sufficient to demonstrate that certain molds, under proper conditions, can do much more than merely spoil a product by rendering it moldy. They, in fact, develop substances with a high degree of toxicity. The present paper will, first, summarize information on a number of mycotoxins which have been isolated, and then explore in some detail the toxic substances produced by A. flavus and designated as aflatoxins.

Many Mycotoxins Reported

Development of toxins is not limited to one or several species of molds. A partial list of the molds, infected material, toxic substances, susceptible animals and symptoms has been compiled by Friedman (12), and is given in Table 1.

An examination of the data in this table leads to a number of conclusions. First, toxin was produced on a variety of substrates. This might be expected since molds can grow on most feeds and foods provided sufficient moisture is present. Second, the toxic material varied in its nature although some of the toxins have not been characterized. Third, many animals and man were susceptible to some if not all of the toxins. Finally, the toxins generally seemed to be rather potent in that they often caused death after ingestion. Many of them also appeared to affect the liver, an organ which is incapable of regen-
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### Table 1. A Summary of Some Data on Mycotoxins

<table>
<thead>
<tr>
<th>Mold</th>
<th>Infected Product</th>
<th>Toxin</th>
<th>Reported Susceptibility</th>
<th>Symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Sclerotinia sclerotiorum</em></td>
<td>Celery</td>
<td>8-Methoxy psoralen</td>
<td>Man, Rabbits, Mouse</td>
<td>Blistering lesions on skin exposed to sunlight</td>
</tr>
<tr>
<td><em>Fusarium sporotrichioides</em></td>
<td>Cereal grains</td>
<td>Unknown</td>
<td>Man, Cat, Guinea pig, Dog, Monkey</td>
<td>&quot;Alimentary toxic aleukia&quot;—Hemorrhages of skin and mucous membranes, necrotic ulcers in oral and pharyngeal tissues, leukopenia, anemia, fever, bone marrow exhaustion</td>
</tr>
<tr>
<td><em>Sporodesmium barkeri</em></td>
<td>Rye grass, Bermuda grass</td>
<td>&quot;Sporodesmium&quot; (C₄H₂₂O₅N₅S₂CR)</td>
<td>Sheep, Cattle, Guinea pig, Rabbit, Mouse</td>
<td>&quot;Facial eczema&quot; in ruminants, hyper-irritability, lacrimation, nasal discharge, photosensitivity, icterus, stenosis, obliteration of bile ducts, cirrhosis</td>
</tr>
<tr>
<td><em>Stachybotrys atra</em></td>
<td>Hay, Straw, Grain</td>
<td>Stable to heat and radiation; destroyed by alkali</td>
<td>Horses, Cattle, Mice, Guinea pig, Dogs, Man</td>
<td>Stomatitis, inflammation of buccal tissues, thrombocytopenia, prolonged clotting time, fever, leucocytopenia, massive hemorrhages, fatal in 3 to 4 weeks, dermal inflammation in man.</td>
</tr>
<tr>
<td><em>Aspergillus chevaleri</em></td>
<td>Hay, Grain</td>
<td>Unknown</td>
<td>Cattle, Mice, Rabbits</td>
<td>Acute—fatal in 4 to 5 days, chronic hyperkeratosis</td>
</tr>
<tr>
<td><em>Aspergillus clavatus</em></td>
<td>Pelleted feed</td>
<td>Unknown</td>
<td>Rabbits</td>
<td>Dermal toxicity</td>
</tr>
<tr>
<td><em>Aspergillus fumigatus</em></td>
<td>Fodder</td>
<td>Unknown</td>
<td>Cows</td>
<td>Hyperkeratosis</td>
</tr>
<tr>
<td><em>Aspergillus flavus</em></td>
<td>Peanuts, Grains</td>
<td>Aflatoxin</td>
<td>Turkeys, Ducks, Swine, Calves, Rats</td>
<td>Liver parenchymal cell damage, bile damage, bile duct proliferation, hepatoma</td>
</tr>
<tr>
<td><em>Aspergillus flavus</em></td>
<td>Corn</td>
<td>Unknown</td>
<td>Swine, Mice</td>
<td>Anorexia, cachexia, icterus, fatal in 1 to 5 days, profuse hemorrhages in all tissues, mortality—25 to 50%</td>
</tr>
<tr>
<td>Penicillium rubrum</td>
<td>Cereal Grains</td>
<td>Unknown</td>
<td>Higher Vertebrates</td>
<td>Ascending paralysis of CNS</td>
</tr>
<tr>
<td>Penicillium toxicarum</td>
<td>Rice</td>
<td>Citrinin</td>
<td>Mice</td>
<td>Acute glomerulonephrosis, liver damage</td>
</tr>
<tr>
<td>Penicillium citrinum</td>
<td>Rice</td>
<td>Rugulosin</td>
<td>Mice, Rats</td>
<td>Fatty degeneration of liver, Kidney damage</td>
</tr>
<tr>
<td>Penicillium rugulosum</td>
<td>Rice</td>
<td>Chloride-containing peptide</td>
<td>Rats</td>
<td>Fatty degeneration of liver, bile duct hyperplasia, focal necrosis and hemorrhages of the liver, primary malignant hepatomas</td>
</tr>
</tbody>
</table>

*As reported by Friedman (12).*

eration. Consequently, changes in this organ are quite permanent in nature and tend to be rather deleterious to the welfare of the animal or human being.

One of the toxins listed in Table 1 is aflatoxin which is the product of *A. flavus* and *Aspergillus parasiticus*, although the latter mold was not mentioned in Table 1. This toxin recently has received more attention than the others since it has been associated with a food crop—peanuts. This emphasis has resulted in the accumulation of a substantial amount of information on aflatoxin. The remainder
of this paper will be devoted to a consideration of some of this information.

Aflatoxins.

It was mentioned earlier that a toxic substance now designated as aflatoxin was first associated with moldy peanut meal which caused the death of large numbers of turkey poults and ducklings after they ingested the feedstuff. The mold recovered from the peanut meal and found able to produce the toxin was Aspergillus flavus. Before considering the nature of the toxin and ways it affects animals, some space will be devoted to a description of the mold responsible for the problem.

Description of Aspergillus flavus. The genus Aspergillus has certain peculiar characteristics which serve to distinguish it from other genera of molds. The vegetative mycelium consists of septate branching hyphae which range from colorless to brightly colored and, in a few instances, are colored in localized areas (20). The reproductive or conidial apparatus develops in the form of conidiophores and heads from specialized, enlarged, thick-walled hyphal cells designated as foot cells. Conidiophores, either septate or nonseptate, usually enlarge at the top to form fertile vesicles that in turn bear fertile cells or sterigmata. Conidia (or spores) which may vary in color, size, shape, and markings are produced from the tips of either the primary or secondary sterigmata. Figure 1 (20) illustrates the major characteristics of molds in the genus Aspergillus as they have just been described.

Aspergillus flavus has all of the characteristics just discussed but is designated as a species because it differs from other molds in the same genus with regard to some of its distinctive features. The principal characteristics of Aspergillus flavus include: (a) the conidia are round or virtually round when mature and may have a rough surface, (b) conidial heads are round to radiate or columnar in shape and are very light yellow-green to jade green or cress green in color, (c) conidiophores are colorless and usually have a rough surface, and (d) vesicles tend to be round and are fertile over most of their surface. These characteristics of Aspergillus flavus are illustrated in Figure 2 (20).

This brief description of the genus Aspergillus and, more specifically, of Aspergillus flavus, is sufficient to provide some background on the type of organism responsible for the formation of aflatoxin. The toxic substance itself will now be considered.
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Figure 3. Structures of aflatoxins B1, B2, G1 and G2 (25).

Chemical and physical nature of aflatoxin. Aflatoxin consists of four components when viewed under ultraviolet light. Two of these components emit blue visible light and are designated as B1 and B2. The other two fluoresce with a yellow-green color and are called G1 and G2 (25). The amounts and relative proportions of these four compounds present in culture extracts are variable, depending on such factors as mold strain, medium composition, and cultural conditions. Typically, aflatoxins B2 and G2 are present in smallest quantities, whereas the concentration of B1 is usually greatest. These four compounds were originally isolated by investigators in England (18, 21) and the Netherlands (24).

The molecular formula of aflatoxin B1 was established as \( C_{17}H_{12}O_6 \) and of G1 as \( C_{17}H_{12}O_7 \), whereas aflatoxins B2 and G2 were found to be the dihydro-derivatives of the parent compounds and have the formulae \( C_{17}H_{14}O_6 \) and \( C_{17}H_{14}O_7 \), respectively (13). Structures based largely on interpretation of spectral data were proposed in 1963 for aflatoxins B1, C1, and B2 (4, 5, 9). These and the proposed structure for G2 are shown in Figure 3.

These closely related compounds are highly substituted coumarins, and thus are among a large group of naturally occurring compounds with many pharmacological activities. It should be pointed out before concluding the discussion on the nature of aflatoxins that all four are very heat stable. The reported melting points for B1, B2, G1, and G2 are 269, 288, 245, and 239°C, respectively (25).

The discussion just completed has served in part, to describe the toxic metabolites of Aspergillus flavus from physical and chemical viewpoints. Attention will now be directed to the effect of these toxins on various animals.

Effects of aflatoxins on animals.

The effect of aflatoxins on animals is governed by:

(a) the dosage administered in the form of moldy feed or in another fashion,
(b) the kind of animal,
(c) the length of time that the animal is exposed to the toxin, and
(d) the age of the animal.

Wogan (25), in a recent review of this subject, approached the problem of the effect of aflatoxin on animals from three points of view: (a) acute toxicity associated with ingestion of a lethal dose, (b) subacute toxicity associated with consumption of small amounts of toxin, and (c) carcinogenic properties of the toxin. The same pattern will be followed in the present discussion.

| Table 2. Pathological Changes in Animals That Received Aflatoxin-Contaminated Feed* |
|-----------------------------------------------|-----------------|----------------|----------------|----------------|----------------|----------------|
| Liver Lesions                                | Calves          | Cattle         | Swine          | Sheep          | Duckling        | Adult Duck     |
| Acute necrosis and hemorrhage                | -               | -              | +              | -              | +              | +              |
| Chronic fibrosis                             | +               | +              | +              | +              | -              | +              |
| Regeneration nodules                         | -               | +              | +              | +              | ±              | +              |
| Bile duct hyperplasia                        | +               | +              | +              | +              | +              | +              |
| Veno-occlusive disease                       | +               | +              | -              | 0              | -              | -              |
| Enlarged hepatic cells                       | +               | +              | +              | +              | +              | +              |
| Liver tumors                                 | 0               | 0              | 0              | 0              | -              | +              |

As reported by Wogan (25).
Acute toxicity. The aflatoxins are acutely toxic to most animal species. Early experimental studies as well as observations in the field suggested that the duckling was the species most susceptible to acute poisoning. The LD₅₀ of one-day-old ducklings is approximately 0.5 mg per kg. This value is considerably smaller than those for the rat and hamster. Some tests indicate that the dog, rabbit, guinea pig, and rainbow trout all have LD₅₀ values similar to that of ducklings (6, 25).

In most species, death usually occurs within 72 hours after the toxin is administered. Examination of animals after death consistently reveals gross liver damage and occasional hemorrhaging in the intestinal tract and peritoneal cavity.

Animals appear to become less sensitive to the toxin as they grow older. For example, a one-day-old rat has an LD₅₀ of 1.0 mg toxin per kg of body weight, whereas after it is 21 days old, the LD₅₀ value has increased to 7.0. As a basis for comparison, the LD₅₀ of lead arsenate is approximately 500.

The structure of the aflatoxin molecule also affects its toxicity. Aflatoxin B₁ is most potent, followed in order by C₁, B₂, and C₂. The presence of the additional oxygen in the C compounds results in a reduction of activity by a factor of two, whereas the unsaturated compounds are approximately 4.5 times as potent as the dihydro-derivatives.

The information just presented becomes a bit more meaningful when it is realized that one of the most toxic peanut meals ever encountered contained approximately 10 ppm. of aflatoxin B₁. As little as 1.0 gram of this meal proved lethal to day-old ducks (21).

In another instance calves died when they were fed some of the original Brazilian peanut meal (17). Later experiments were conducted in which other calves received diets containing 18% of a highly toxic peanut meal (3). The calves became unthrifty and died within 16 to 25 weeks. The same toxic meal, when fed to three-to-four-year-old dairy cattle in a diet containing 20% of the meal, caused a loss of condition. Cows aged eight to ten years showed no clinical symptoms when they received the toxic meal at the same level.

Subacute toxicity. Animals which consume sublethal quantities of aflatoxin for several days or weeks develop a subacute toxicity syndrome which commonly includes moderate to severe liver damage. Several types of liver lesions have been observed in different species, and this information is summarized in Table 2 (25). Consideration of the data leads one to conclude that sheep are rather resistant to effects of the toxin, and that biliary hyperplasia (a condition in which there is excessive growth of liver tissue) is the lesion most consistently observed in all species except sheep (25).

Subacute toxic effects of aflatoxins in monkeys have been reported (23). In the experiments young Rhesus monkeys (1.5 to 2.0 kg) were fed either 1.0 mg of aflatoxin per day or 0.5 mg per day for the first 18 days followed by 1.0 mg per day. All animals lost their appetite and died in 14 to 28 days. The principal findings on autopsy included liver lesions similar to those seen in ducklings and which were suggestive of liver cirrhosis.

Carcinogenic properties of aflatoxin. Prolonged administration of the toxin at subacute levels leads to formation of liver tumors which are cancerous in nature. This was observed in early investigations on the feeding of toxic peanut meal to rats (16). After feeding a purified diet containing 20% of toxic peanut meal for six months, nine of eleven rats developed multiple liver tumors, and two of these displayed lung metastases. The carcinogenicity of toxic peanut meal has been demonstrated repeatedly since then, and aflatoxin has clearly been shown to be the responsible agent.

Precise dose-response conditions have not yet been established, but some information is available regarding relationships between tumor incidence in rats and aflatoxin content of contaminated peanut meals. Results of several studies have demonstrated a good correlation between liver tumor incidence and dietary aflatoxin in the range of 0.06 to 1.8 ppm (19). Administration of the highest level (e.g., 1.8 ppm) for 370 days was accompanied by a tumor incidence in excess of 90%. The lowest level of toxin studied (0.005 ppm) failed to induce liver tumors within a similar time period.

Data accumulated from feeding tests employing the pure toxin have permitted the estimation of the effective dose of aflatoxin B₁ for the induction of liver tumors in rats. It has been estimated that this dose is approximately 10 µg per day (8). When this value is compared with similar estimates for other hepatocarcinogens such as dimethylnitrosamine (750 µg/day) and butter yellow (9,000 µg/day), the relative potency of aflatoxin is readily apparent.

The rainbow trout was found to be considerably more sensitive than the rat to the carcinogenic effects of aflatoxin. It has been shown that this fish develops liver tumors at significant rates when fed purified diets containing only 0.5 to 2.0 µg aflatoxin B₁ per kg (i.e., 0.5 to 2.0 ppb) (6, 7, 22). The apparent sensitivity of this fish has suggested that aflatoxin may be an etiological agent of the so-called "trout hepatoma syndrome."

Metabolic alterations of aflatoxin.

The discussion on aflatoxins, up to this point, has been concerned largely with the effect of the toxin on a number of animals. There is another side to
the liver. The nature of compounds present in urine cludiJ;lg the story—the changes that may result in the toxin as a result of its metabolism by animals. Before concluding the discussion, this aspect of the problem will be briefly considered.

Studies with rats and radioactive labeled aflatoxin indicated that 25 to 30% of the toxin was metabolized to CO2, 25% was excreted in the urine, 25% was contained in feces, and six to nine per cent appeared in the liver. The nature of compounds present in urine and feces has not been determined, and the metabolic pathways are not fully understood (25).

In another series of tests, rats were fed a dried, heat-treated culture of A. flavus grown on peanuts and also some pure aflatoxin B1 (11). Chromatographic analysis of an extract of milk produced by the rats revealed the presence of a component different from aflatoxin, but one which retained the toxic properties of the mycotoxin. It was concluded that the lactating rat can convert aflatoxin B1 to another still toxic form and secrete it in the milk. Similar observations have also been made on dairy cattle (1, 2, 11). In fact, it has been demonstrated that the toxic component in cow's milk is associated with casein and remains with the milk protein when it is precipitated with rennin remains with this milk component. Tests on another product of animal origin, namely eggs, revealed the absence of a toxic substance even when the hens that produced the eggs received a diet containing 15 per cent toxic peanut meal (2).

**Summary**

Production of toxic metabolites has been associated with the growth of different molds on a variety of substrates including cereal grains, celery, peanuts, hay, and straw. Most of the toxins affect more than one species of animal, and many of them induce pathological changes in the liver.

Most research attention has been devoted to the heat stable aflatoxin produced by Aspergillus flavus. Actually, four different aflatoxins are produced. All are highly substituted coumarins and two of them (B1 and B2) fluoresce with a blue color under ultraviolet light, whereas the others (G1 and G2) fluoresce with a yellow-green color.

The aflatoxins are acutely toxic to most animal species and death will result if enough is ingested, especially when the animal is young. When low levels of the toxin are consumed for several days or weeks, symptoms of subacute toxicity develop. These include biliary hyperplasia and hepatomas. A daily intake of 10 µg of toxin appears adequate for the induction of hepatomas.

Aflatoxin ingested by animals undergoes certain metabolic changes within the animal body. Rats excrete some of the toxin as CO2, some in the urine, and some in feces. Approximately 6-9% of the ingested toxin is retained in the liver. Rats and daily cattle have also been found able to modify and excrete some of the toxin in milk. The milk toxin is associated with the casein fraction and on precipitation by rennin remains with this milk component.

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ASSOCIATION AFFAIRS

MISSOURI AWARDS TO BAIRD
AND NICKEL

The Missouri Association of Milk and Food Sanitar­
ians at its awards dinner in connection with the 35th
Annual Milk and Food Sanitation Conference at Col­
bumbia, April 10-12, 1967, selected I. H. Baird for the
1967 Sanitarians Award and presented to Vernon
Nickel a 25 year certificate and pin for continuous
service to the Association.

I. H. Baird, D.V.M., Director of Laboratory and
Milk Control for the St. Joseph Department of Health,
has given 45 years service to the health and welfare
of his community. First employed as City Bacteri­
ologist he was instrumental in the adoption of the
first milk ordinance in St. Joseph in 1929 and by con­
tinued revisions and modifications has kept the city’s
milk program at the highest level.

An outstanding achievement was the initiation of
the first mastitis control program in the state. Start­
ing without guidelines and no standardized tests,
“Doc” devised a program to suit his needs. He in­
troduced test methods, particularly for subclinical
mastitis, sometimes over the objections of local vet­
erinarians but his program is now fully supported
in the St. Joseph area. He has been an active member
of state public health and veterinary medical associa­
tions and his work in St. Joseph has contributed sub­
stantially to the formation of the Missouri Mastitis
Council.

Vernon D. Nickel, with the St. Louis City Health
Division, has long been active in the affairs of the
Missouri Association and is presently chairman of its
Dairy Farm Methods Committee. “Nick” is also ac­
tive nationally, serving as the chairman of the Sub­
committee on Education of the IAMFES Dairy Farm
Methods Committee. His group is responsible for
the selection of outstanding farm publications cur­
rently being abstracted in the Journal.

The 35th Conference was well attended and an in­
teresting and informative program was presented,