MYCOTOXINS

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INTRODUCTION

Mycotoxins are secondary mold metabolites that produce toxic reactions in animals or people exposed to them. Although it is not a novel concept that fungi are able to produce biologically active metabolites, the fact that some food spoilage molds can inject such substances into the food supply has come to be widely appreciated only within the last two decades. Mass outbreaks of overt poisoning by moldy food has been documented in only a few instances in human populations, but somewhat more frequently in domestic animals. Historically, ergotism caused by scabrous grains was recognized during the Middle Ages, and although large-scale episodes are now uncommon in man, small outbreaks still occur (1), and the toxicosis is frequently seen in domestic animals (2). In only one other instance is there extensive documentation of mycotoxin poisoning in humans. This is a syndrome known as alimentary toxic aleukia (ATA), a pan-leukocytopenia that occurred in the USSR during the later years of World War II (3).

A mycotoxicosis caused by compounds eventually to be called aflatoxins was first reported in 1960–1961, and this particular problem had a number of economic and health-related impacts that served to focus much wider attention onto the field as a whole. The toxicosis affected large numbers of animals, in this instance, poultry flocks, causing significant economic losses. The toxic substances were present in peanuts, a commodity of considerable economic importance in international trade, primarily for its oil content. The residue following oil removal is a concentrated source of nutritionally high-quality protein that finds application not only in animal feeds, but also in the formulation of protein-supplement foods distributed by international agencies. Perhaps the most crucial findings in the evolution of knowledge about the problem were the discoveries that the toxic substances were also powerful carcinogens, and that they were produced by a widely disseminated and common food-spoilage mold.

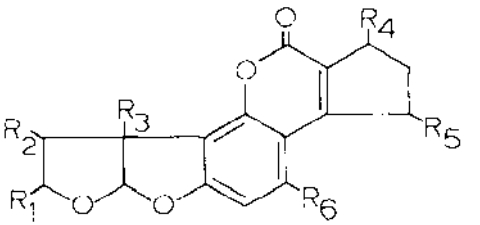
Under the combined influence of these factors, many aspects of the general mycotoxin problem came to be investigated. Expertise of many disciplines, including microbiology, chemistry, agriculture, toxicology, pathology, epidemiology, and medicine have at various times been brought to bear on the general problem. By

virtue of the large literature that has consequently accumulated, it would be impossible to attempt a comprehensive survey. This review is intended rather as a report on the current status of the field. It presents a résumé of areas thought to be of most clearly defined public health relevance to man, or examples illustrative of the general character of the mycotoxin problem currently under active investigation. Further details are available in a recently published comprehensive survey of the entire field (4–6).

AFLATOXINS AND STERIGMATOCYSTINS

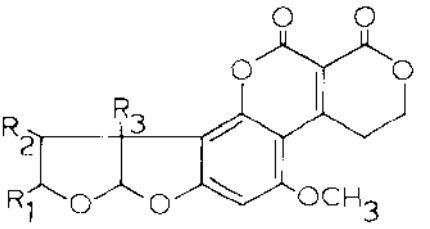
More information is available about aflatoxins than any other single group of mycotoxins. Most of this large literature is beyond the scope of this review, which emphasizes recent developments, mainly in the areas of biological effects, metabolism, and mode of action. Various facets of the field have been subjects of previous specialized reviews (e.g. 7–9), and the entire subject has been comprehensively surveyed in monograph (10) and summary form (11). Those sources give detailed references to the original literature on which the following general summary is based.

Chemically, the aflatoxins are highly substituted coumarins and contain a fused dihydrofurofuran configuration, which is peculiar to a limited number of compounds of natural origin. The compounds occur in two series, aflatoxin B_1 and derivatives, and aflatoxin G_1 and derivatives. Structures of presently known aflatoxins from biological sources are shown in Figures 1 and 2. Sterigmatocystin and its



Aflatoxin	R_1	R_2	R_3	R_4	R_5	R_6
B_1	H	H	H	=O	H	OCH_3
B_2	H_2	H_2	H	=O	H	OCH_3
B_{2a}	HOH	H_2	H	=O	H	OCH_3
M_1	H	H	OH	=O	H	OCH_3
M_2	H_2	H_2	OH	=O	H	OCH_3
P_1	H	H	H	=O	H	OH
Q_1	H	H	H	=O	OH	OCH_3
R_0	H	H	H	OH	H	OCH_3

Figure 1 Aflatoxin B_1 and its derivatives.



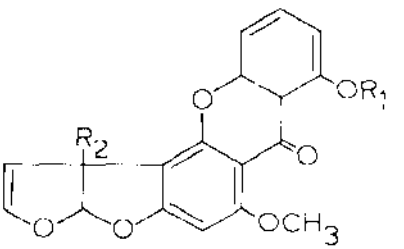
Aflatoxin	R_1	R_2	R_3
G_1	H	H	H
G_2	H_2	H_2	H
G_{2a}	OH	H_2	H
GM_1	H	H	OH

Figure 2 Aflatoxin G_1 and its derivatives.

derivatives are shown in Figure 3. These metabolites are included here in order to indicate their structural similarities to the aflatoxins.

Aflatoxins B_1, B_2, B_{2a}, M_1, G_1, G_2, and G_{2a} have been isolated from mold cultures and/or from contaminated foods. Certain of these, as well as the remaining derivatives whose structures are shown are metabolites produced from the parent toxins, by animals or animal tissues, as discussed subsequently. Sterigmatocystin and its derivatives have been identified mainly in mold cultures and in some instances in moldy grains.

The aflatoxins are produced by a few strains of *Aspergillus flavus* or *A. parasiticus*, fungi whose spores are widely distributed, especially in soil. Those organisms that are capable of toxin production generally synthesize only two or three aflatoxins



R_1	R_2	
H	H	Sterigmatocystin
CH_3	H	O-Methyl "
CH_3	OH	Aspertoxin

Figure 3 Sterigmatocystin and its derivatives.

under a given set of conditions. When they occur as food contaminants, aflatoxin B_1 is always present. Although aflatoxins B_2, G_1 and G_2 have also been reported in contaminated products, they generally occur less frequently than B_1, and have never been reported in the absence of B_1. This is an important point, because B_1 has the highest potency of the group as a toxin and as a carcinogen.

With respect to substrate, requirements for toxin are relatively nonspecific, and the mold can produce the compounds on virtually any food (or indeed on simple synthetic media) that will support growth. Thus, any food material is liable to aflatoxin contamination if it becomes moldy. However, experience has shown that the frequency and levels of aflatoxins found varies greatly among foods collected in a given region and in different regions.

Sterigmatocystins are metabolites of *Aspergillus versicolor, A. flavus, A. nidulans, A. rugulosum,* a *Bipolaris* species, and a *penicillium.* Although a chemical assay for their detection is available, little information has as yet been published about their occurrence in foods.

The toxicity, carcinogenicity, and biochemical effects induced in biological systems by aflatoxins have been reviewed in detail recently (7, 11), and only a brief summary is necessary here to indicate the present status of these facets of the field. Acute or subacute poisoning can be produced in animals by feeding aflatoxin-contaminated diets or by dosing with purified compounds. Although there are wide species differences in responsiveness to acute toxicity, no completely refractory species is known. Symptoms of poisoning are produced in most domestic animals by aflatoxin levels in the feed of 10 to 100 mg/kg (ppm) or less. As regards lethal potency to experimental animals, the oral or parenteral LD_{50} values are generally in the range of 5 to 15 mg/kg body weight for aflatoxin B_1. The value for trout, the most sensitive species, is < 0.5 mg/kg, and that for the mouse, the least sensitive, is 60 mg/kg.

In both acute and subacute poisoning, the liver is the main target organ for aflatoxin B_1. The chief pathologic lesions produced in liver are periportal or centrilobular necrosis, bile duct proliferation, and, in some species, cirrhosis. Repeated dosing or chronic feeding induces hepatocellular carcinoma and cholangiocarcinoma in many animal species, and also renal adenocarcinomas in some rat strains. With respect to carcinogenic potency, aflatoxin B_1 is the most potent liver carcinogen yet discovered. As in susceptibility to acute poisoning, there is a rather wide variation among species in their carcinogenic response. However, hepatic carcinogenesis by aflatoxin B_1 has been shown experimentally in trout, salmon, duck, rat, mouse, Rhesus monkey, and marmoset. Trout and one rat strain develop liver tumors in response to continuous feeding of diets containing 1 mg/kg (ppm) aflatoxin B_1. Susceptibility to carcinogenic effects can be modified by age, sex, microsomal enzyme induction, liver insults of various types, and certain nutritional deficiencies.

Many of the biochemical changes induced in various biological systems by aflatoxin B_1 follow a consistent pattern. Administration of the toxin to rats is quickly followed by pronounced inhibition of DNA and RNA polymerases, and similar

responses have been observed in human and animal cell cultures. Protein synthesis is also impaired, particularly under conditions where synthesis is strongly influenced by alterations in RNA synthesis. Available evidence indicates that polymerase inhibition is an indirect consequence of impaired template activity of chromatin subsequent to toxin-chromatin interaction. Consequently, interactions between aflatoxin or some derivative of it with DNA or other component of chromatin is viewed as the initiating event in the observed series of reactions. Much of the current literature deals with one or another facet of this sequence of biochemical events. However, another line of evidence that may be related to the mode of action of the toxins deals with their ability to interact with membranes of the endoplasmic reticulum and thereby alter polysomal binding to those membranes. However, the available evidence is as yet inadequate to explain in detail the biochemical basis of the cytotoxicity and carcinogenic effect of these toxins.

Considerable attention has been focused on the metabolic fate of aflatoxin B_1, research in this area being intended mainly to produce data bearing on mechanisms that might explain mode of action and differences in susceptibility to the toxin among various animal species. Information generated to date is inadequate to provide such an explanation, but some general outlines of metabolic patterns are beginning to emerge (12, 13).

Patterns of tissue distribution and excretion of aflatoxin B_1 following oral or parenteral dosing have been studied in several species. Experiments with ^{14}C-labeled toxin indicated that more than 90% of a single dose is excreted within 24 hr by rats (7, 12). Feces represents the principal excretory route, accounting for up to 75% of the dose with urine containing an additional 15–20%. Retained radioactivity is present mainly in liver. This pattern of tissue distribution and excretion is generally similar in mice (12), in Rhesus monkeys given a single dose intraperitoneally (14) or orally (15), and in chickens dosed repeatedly by intubation (16, 17). In all of these experiments, the identity of the radioactive material retained or excreted is largely unknown, except that unmetabolized aflatoxin B_1 does not account for more than 5% of the total in any instance. In a few cases, major metabolites have been isolated and identified chemically.

All of the metabolic transformations of aflatoxins B_1 and G_1 known to take place in animals are indicated by the structural derivatives in Figures 1 and 2. The identity of some metabolites has been established from compounds isolated from in vivo sources; others have been produced in vitro. Most of the work has been done on aflatoxin B_1, but in a few cases parallel pathways have been shown for G_1 as indicated in Figure 2.

Hydroxylated derivatives of aflatoxin B_1 are formed through several routes. Ring hydroxylation at the 4 position, producing M_1, appears to be a common pathway. This derivative has been found in milk, tissues, and urine of animals and people ingesting B_1, and is also produced in vitro by liver microsomes of birds, rodents, and primates, including man (13). Ring hydroxylation of the carbon atom β to the carbonyl function of the cyclopentenone ring to form aflatoxin Q_1 was recently discovered in monkey liver preparations (18) and represents the major in vitro

conversion by human liver microsomes (19). This pathway seems to be of only minor importance in rodent and bird liver (13), and free aflatoxin Q_1 has not yet been found in tissues or excreta of any animal exposed to B_1 in vivo.

Aflatoxin P_1, a phenolic derivative, is produced by O—demethylation of B_1. Although this metabolite is a major excretory product in monkeys (14), the free phenol is only a minor in vitro metabolite of liver microsomes of monkeys, mice, and rabbits, and is apparently not formed by other species in vitro.

A further pathway leading to a hydroxylated derivative is the hydration of the 2,3-vinyl ether double bond producing the hemiacetal called B_{2a}. This transformation is accomplished readily by liver microsomes of the rabbit, guinea pig, mouse, chick, and duck, but much less efficiently by the rat (13). Nonenzymatic addition of water also occurs readily under strongly acidic conditions.

All of the preceding metabolic transformations have been demonstrated with crude and purified microsomal preparations. An additional conversion leading to a hydroxylated derivative involving an $NADPH_2$-dependent soluble enzyme is the reduction of the cyclopentenone function of aflatoxin B_1 to produce cyclopentenol called R_0 (or aflatoxicol). This pathway is especially prominent in the livers of rabbits and several avian species, and the reaction is blocked by 17-ketosteroid sex hormones, suggesting that a soluble $NADPH_2$-linked 17-hydroxysteroid dehydrogenase may be involved (13).

It must be emphasized that current knowledge about aflatoxin metabolism is very incomplete. All of the available information on the relative importance of known pathways is at best semiquantitative in character. This is true because only unconjugated metabolites extractable from excreta or incubation media by organic solvents have thus far been identified. Whenever quantitative experiments using radioactive toxin have been done, thus permitting accurate monitoring of all substrate and product fractions, it has been found that significant portions, often a majority, of metabolites are not recovered by solvent extraction and thus remain unidentified. These undoubtedly include, among other possible derivatives, conjugates of known metabolites; all such components will remain unidentified until methods are developed for their isolation. Under these circumstances, speculation based on the toxicologic importance of pathways only partially characterized (e.g. 13) are obviously uncertain and may result in misleading conclusions.

Recent evidence for an additional pathway seems particularly important to the question of metabolic activation of aflatoxin B_1. Rodent liver microsomes convert the toxin to its reactive 2,3-oxide, which has been trapped as an RNA-adduct (20, 21). This system generates a product lethal and mutagenic to bacteria (22), presumably through the production of an electrophilic derivative that reacts with cellular nucleophiles. Production of a derivative lethal to bacteria, presumably through a similar pathway, has also been observed in trout liver (23). Although the epoxide itself has not yet been isolated owing to its great reactivity, a more stable model compound, aflatoxin B_1-2,3-dichloride has been synthesized (24). This electrophilic analog of the epoxide is more potent than the parent toxin in several biological and chemical assay systems. Thus it seems likely that the epoxidation pathway may represent an important activation step in aflatoxin metabolism.

The aflatoxins provide an interesting set of structural homologues for investigations of relationships between chemical structure and biological activity (7). These relationships have not been systematically examined with respect to all biological and biochemical effects of the compounds, but enough work has been done to permit some general statements about this point.

Relatively few comparative carcinogenesis experiments have been done with aflatoxin other than B_1; those that have can be summarized as follows (Figures 1 and 2). In rats, aflatoxin G_1 induces liver tumors similar to those induced by B_1, but with considerably reduced potency, and the same pattern of responses is observed in rainbow trout. In addition to liver tumors, kidney tumors are also induced in rats by G_1. Aflatoxin G_2 is inactive in trout and has not been tested in other species.

Aflatoxin B_2 induces liver tumors in trout with the same potency as G_1, but in rats the dose level required to induce liver tumors is about 150 times the effective dose of B_1.

Aflatoxin M_1 is hepatocarcinogenic to rainbow trout, with about 30% of the potency of B_1. It is also carcinogenic to rats (7), but has a greatly reduced potency than B_1 in that species.

Data regarding various toxic and biochemical actions of aflatoxins consistently indicate a structure-activity series with decreasing potency in the order $B_1 > G_1 > B_2 > G_2$. This generalization holds for toxicity to ducklings, rainbow trout, zebra fish larvae, chick embryos, and cultures of various mammalian cells in vitro. A similar order of potency has also been described for their ability to bind to DNA, alter biochemical processes in vitro, and induce functional changes in rat liver.

Collectively, this information indicates that two functionalities of the aflatoxin molecule are important determinants of its biological activities. Substituents fused to the coumarin nucleus determine activity to the extent that the G configuration is less potent than the B (Figures 1 and 2). Further indication of the importance of this part of the molecule is the fact that R_o is less potent than B_1. Even stronger evidence exists for the importance of the dihydrofurofuran segment of aflatoxin B. Compounds lacking this portion of the molecule are inactive in every system tested. Moreover, reduction of the double bond of the terminal furan ring (B_1 vs B_2; B_2 vs B_{2a}) brings about significant reduction of potency in most systems. Those findings are consistent with the postulated importance of epoxidation as an activation mechanism. On the other hand, hydroxylation in the 4 position (B_1 vs M_1) appears not to affect acute toxicity, but significantly reduces carcinogenic potency for rats.

For the most part, structure-activity relationships established in vitro agree well with those obtained in vivo. Continued investigations of both types should ultimately reveal the cellular and molecular bases of action of those toxins.

TRICHOTHECENE MYCOTOXINS

Toxins in this group comprise a family of closely related sesquiterpenes, all of which are derivatives of a trichothecane ring system, so named for trichothecin, the first member of the group to be isolated. The chemistry and other aspects of this group of compounds have been authoritatively reviewed (25), and only the main points are

mentioned here. The generic structure in Figure 4 shows the structural features shared by all members of the group. The naturally occurring toxins are all esters of parent alcohols, most of which bear only hydroxyl substituents. Two are 8-keto derivatives, and one has a second epoxide in the 7,8 position. More than twenty naturally occurring derivatives of these parent structures have been isolated and identified (25–27).

Trichothecenes are produced by many species of molds in the genera *Fusarium, Myrothecium, Trichothecium, Trichoderma,* and *Cephalosporium.* From the standpoint of food contamination, the fusaria are probably most important (28,29), because they frequently cause mold spoilage of cereal grains. The other genera have more limited distribution with respect to food crops, but toxin-producing strains of all species have a world wide distribution.

A broad spectrum of biological responses has been described for various members of this group of toxins. They were first investigated because of their antifungal and phytotoxic effects (30). Observations that toxin-producing strains of *Fusarium tricinctum* were often isolated from moldy corn and other cereals (31) led to extensive studies on toxic substances produced by that organism and others in the same genus. A highly substituted derivative given the trivial name "T-2 toxin" was identified as a principal toxic metabolite (32), and much of the more recent toxicological literature deals with effects of that compound.

In general, the trichothecenes possess similar biological properties in given bioassay systems, but potency in producing effects varies considerably with structural modification. Groups of compounds with similar substituents on the tetracyclic nucleus have almost identical biological properties. The earliest studies in this area dealt with antibiotic properties of these substances. As a group, they have little or no antibacterial activity, but some are potent antifungal agents. One, trichodermin, is a very potent inhibitor of the pathogenic yeast, *Candida albicans,* and has been proposed as a clinically useful therapeutic agent by virtue of its low mammalian toxicity.

When administered to animals, trichothecenes induce a wide range of toxic responses. All produce dermal toxicity upon direct contact. At low doses, a single application results in a prolonged inflammatory response, and higher levels are necrotizing. This response has been observed in laboratory workers accidentally exposed to the toxins as well as in animals, and is sufficiently characteristic and

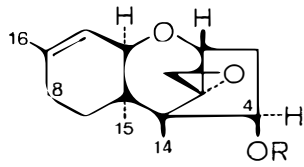


Figure 4 4-β-hydroxy(acyloxy)-12,13 epoxy-Δ⁹-tricothecene (Generic structure of tricothecene mycotoxins).

sensitive to provide the basis for a bioassay for detection and quantitation of the toxins. In one recent version of the assay, rat skin responds to as little as 50 ng of T-2 toxin (33).

Several kinds of clinical and pathological responses have been described following oral or parenteral administration of these mycotoxins to animals. With respect to lethal potency, LD_{50} values in mice and rats are in the range of < 1 to 15 mg/kg for most members of the group; among the least toxic is trichodermin, with an LD_{50} of 500 mg/kg. Fatally poisoned animals generally die later than 12 hr after dosing, but the locus of the toxicity is unknown.

Subacute or chronic dosing of various trichothecenes has been associated with the production of a variety of responses. These have included: lesions of the oral cavity (34), emesis (35, 36), gastrointestinal inflammation and hemorrhage (37), and petechial and ecchymotic hemorrhages in various tissues (25). A particularly prominent effect is a radiomimetic response involving suppression of blood-forming and other rapidly proliferating tissues, with subsequent development of granulocytopenia, anemia, and impaired immune response (25). In chronic feeding studies, trout tolerated a dietary level of 0.4 ppm T-2 toxin for 12 months and rats, 10 ppm for 8 months without adverse effects. No evidence of initiating or carcinogenic activity for rat or mouse skin was found following treatment with T-2 toxin and croton oil (38).

A number of trichothecenes have been shown to be cytotoxic or cytostatic to many kinds of animal and plant cells in culture (25). Cytopathogenicity has been observed in hamster kidney cells, chick embryo fibroblasts, and rabbit reticulocytes; and also in a variety of tumor-derived cells including KB (human epidermal carcinoma), L 1210 (leukemia), Ehrlich ascites, Walker carcinoma, and Yoshida carcinoma. The toxins seem to affect selectively dividing cells, in which they induce lesions similar to those produced by ionizing radiation. The most potent of the compounds have ED_{50} values in the order of 0.5–1.0 ng/ml in the culture medium.

The precise biochemical lesions leading to cell death induced by the toxins remain unidentified. However, a number of investigations have produced evidence of effects on protein synthesis and other cellular processes. One extensive survey revealed that twelve trichothecene mycotoxins inhibited, to varying degrees, incorporation of amino acid into protein by intact reticulocytes (39). This effect was also observed in Ehrlich ascites cells as was inhibition of thymidine incorporation into DNA. Impaired protein synthesis was associated with marked polysomal disaggregation in these systems and was also observed in mouse fibroblasts (40). A specific locus for the effect in mammalian cells has recently been proposed (41). Evidence was presented that suggests that trichodermin inhibits protein synthesis in such cells by blocking on the ribosome the interaction of the peptidyl transferase with the release factor required for chain termination. It remains to be seen whether this effect will prove to be a common site of action responsible for the toxic reactions of this class of compounds.

Structure-activity studies have revealed that the free alcohol derivatives of the naturally occurring trichothecenes are less active than the intact esterified molecules in terms of antifungal, phytotoxic, and dermatitic agents (25). However, they are as active as the esters when administered parenterally or orally to animals, suggest-

ing that the difference may be due to cell penetration rather than inherent toxicity of the molecular fragment. Additional evidence is available to suggest that toxic potency is dependent upon the 12,13 epoxy group, the presence of the 9-ene, and esterification of several of the hydroxyl substituents (42).

It has not been established unequivocally that trichothecene mycotoxins have been the cause of any given outbreak of a mycotoxicosis in animals or man. However, an impressive amount of circumstantial evidence suggests that toxins of this class might very well have been involved in both past and present episodes of such syndromes.

The circumstantial case linking these toxins to alimentary toxic aleukia in man is particularly suggestive. The disease is associated with the ingestion of grain (wheat, rye, oats, millet, or buckwheat) infested with fungi, most importantly *Fusarium poae* and *F. sporotrichioides* (3). Signs of the toxicosis in humans include fever, hemorrhagic rash, bleeding from mucous membranes, necrotic angina, leukopenia, agranulocytosis, and sepsis. Large-scale episodes involving up to 10% of the population of certain districts have occurred in the USSR.

Fungal extracts originally identified as probable causative agents in the syndrome were thought to contain toxic steroidal glycosides. However, a sample of one such extract ("poaefusarin") was recently found to contain the trichothecene T-2 toxin in sufficient amounts to account for its toxicity (43). In addition, the fungi associated with the toxicosis, notably *F. tricinctum* are known to produce trichothecenes in laboratory conditions (0°C) approximating those important in the field outbreaks. The toxins themselves have chemical properties and toxicological activities that resemble the toxic agents causing the disease in man.

Similar evidence suggests that these mycotoxins might also have been involved in the etiology of several types of toxicosis of animals. These include so-called moldy-corn toxicosis of pigs, poultry, and cattle and stachybotryotoxicosis of horses and other domestic animals. Evidence linking the trichothecenes with these and other syndromes has recently been summarized (44).

The potency and stability of these mycotoxins and the ubiquitous nature of the fungi that produce them combine to stress their importance as potential contaminants of the food supply. Apart from the dramatic overt poisoning episodes outlined above, little is known about the frequency with which they occur in foods or the consequences of low-level contamination if it does occur. Analytical methodology for detecting trichothecenes in foods is almost entirely lacking, and it will be impossible to make a realistic assessment of their significance until such methodology is developed and applied in surveillance programs.

CURRENT TRENDS IN MYCOTOXIN RESEARCH

Both aflatoxins and trichothecenes continue to be actively investigated. Other facets of the field are also under study, and some selected examples will serve to illustrate the general lines along which such research is progressing.

One approach being pursued mainly for purposes of quality control and surveillance is the development and application of analytical methods for detecting the

presence of known mycotoxins in important food commodities. Methods at different stages of applicability are available for mycotoxins of various classes (45), and additional ones are being developed (46). As these are applied to foods, it is not uncommon to find occasional samples contaminated by mycotoxins. For example ochratoxin A has been discovered in some samples of wheat, oats, barley, corn, and rye; in one instance, citrinin and sterigmatocystin were simultaneously present (47). Similarly, patulin, a lactone with carcinogenic properties produced by *Penicillium expansum* and other molds, has been found in apple juice at levels up to 150 mg per liter (48). Additional surveys of this type will no doubt reveal more about the types of mycotoxins that actually occur in foods and the conditions favoring contamination. Hopefully, such information will lead ultimately to successful control measures to minimize contamination.

A second avenue of approach is to isolate fungi from foods and determine their capacity for producing known or unknown mycotoxins when cultured experimentally. Many such surveys have been done, and two recent studies will illustrate the character of findings generaliy obtained. Among a total of 163 molds isolated from pecans, 6 isolates were sterigmatocystin producers, and 1 produced aflatoxins (49). In a similar study on wheat flour and bread, among a total of 70 fungal isolates, 9 penicillia produced penicillic acid, 2 produced patulin, and 4 produced luteoskyrin. One *Aspergillus ochraceous* produced both ochratoxin A and penicillic acid (50).

In a survey of Thai foods for mycotoxin-producing fungi, more than 50 isolates were identified which produced toxic metabolites exclusive of known mycotoxins (51). In follow-up studies on these organisms, one *Aspergillus clavatus* isolated from rice was shown to produce cytochalasin E (52) and two tremorigenic toxins of unestablished structures (53). This type of study together with those previously alluded to indicate potential hazard from mycotoxin contamination, but do not demonstrate actual risk.

A third line of endeavor currently being followed involves attempts to identify the toxic agents produced by fungi incriminated in outbreaks of moldy food poisoning of poultry or livestock. One such approach involves studies on *Penicillium viridicatum,* an organism frequently isolated from grain that is toxic to swine and other animals. Various isolates of the organism are known to be capable of producing one or more known mycotoxins including ochratoxins, citrinin, oxalic acid, and penicillic acid as well as hepatorenal and photosensitizing toxins of unknown structure (54). Current research activities include attempts to characterize the toxic responses to culture extracts (55–57) to optimize toxin production (58), and to identify new mycotoxins (59, 60). One recent report (61) suggests that some component of culture extracts of *P. viridicatum* may be carcinogenic to mice upon prolonged feeding, but the active components have not been identified.

SIGNIFICANCE OF MYCOTOXINS TO HUMAN HEALTH

Despite the availability of this relatively large scientific literature, it is still difficult to make a rcalistic assessment of the public health significance of mycotoxins. In

respect to their potential health hazards, it is possible to categorize the various known mycotoxins and mycotoxicoses as follows, according to the status of current knowledge:

1. Two toxicoses in man are known by virtue of direct evidence of exposure and response to have been caused by mycotoxins. This category would include only ergotism and alimentary toxic aleukia; possibly aflatoxins will be added to the list as additional information becomes available.

2. Some mycotoxins known to be toxic to animals have been identified in human foods by chemical assay. However, little or no direct evidence is available on the extent to which contaminated foods are actually eaten or whether toxic responses are induced in man. This category would include aflatoxins, sterigmatocystin, ochratoxins, patulin, and penicillic acid.

3. A few specific mycotoxins are known to occur in the feeds or forage of domestic animals and to cause toxicity syndromes in them. These agents would present the additional potential risk of human exposure through residues in meat, milk, or eggs. In addition to aflatoxins, ochratoxins, trichothecenes, and zearalenone, this list would include citrinin and sporidesmin.

4. Numerous fungi isolated from human or animal foods or feedstuffs can be shown to be toxigenic when cultured experimentally. It is generally unknown whether the potential for toxin production is ever actually expressed. However, in a few instances, specific mycotoxins in this category have been identified, e.g. cyclopiazonic acid, cytochalasin E, rubratoxins, and a butenolide.

These lists will no doubt enlarge with additional research, especially on the laboratory identification of additional toxins and toxinogenic molds. However, accumulation of additional information on human exposure and response will be much more difficult, owing mainly to the necessity to develop reliable chemical assay methodology for food analysis. Although sensitive and accurate methodology has been developed for aflatoxins and a few other mycotoxins, in many instances no analytical methods have been worked out and development of them presents formidable chemical challenges.

Direct evidence for assessing public health significance would require a knowledge of both level of exposure (i.e. actual intake) and the association of that exposure with incidence of a specific disease entity. While such an association has not been established for any mycotoxicosis in man, the most serious attempts in this regard have been studies relating aflatoxin exposure to liver cancer incidence. The results of four such surveys are pertinent. The geographical distribution of aflatoxins in peanuts was compared with liver cancer incidence data in Swaziland (62). It was found that areas with high disease incidence also showed greater contamination of peanuts by aflatoxins, and the eating habits of people in the high cancer areas seemed to provide greater opportunity for aflatoxin exposure. In a more extensive study in Uganda (63) it was found that nearly 30% of food samples contained aflatoxins, some with levels exceeding 1 ppm. The geographical distribution of liver cancer incidence was such that the areas with highest disease incidence coincided with regions with most frequently contaminated foods. Neither of these studies provided quantitative data on aflatoxin intakes.

Two additional surveys, one in Thailand and one in Kenya, have provided such data. Aflatoxin contamination of Thai foods and foodstuffs was measured (64), and daily aflatoxin consumption was determined (65). Simultaneously, liver cancer incidence was measured directly in a population of 200,000 (66). Aflatoxin ingestion among subpopulations varied from 2 ng/kg per day to 80 ng/kg per day, and liver cancer incidence was increased by threefold in the high intake group. In the Kenya study, aflatoxin consumption was also measured directly, and cancer incidence was determined from cancer registry data. Intake and incidence values were of the same order of magnitude as the Thai data and were positively associated with each other. Collectively, these data indicate a higher risk for liver cancer in populations consuming aflatoxin-contaminated foods.

Quantitative cause-effect relationships have not been established in the case of aflatoxins or any other mycotoxin, and the difficulties involved in collecting the necessary data in human populations make it unlikely that this will be accomplished in the foreseeable future. Therefore, in order to minimize the risk of mycotoxin contamination of the food supply, prudence requires that effective control measures be implemented to minimize accidental mold damage of foods or food raw materials and to demonstrate the absence of toxic products in foods involving fermentation by molds.

Even with the implementation of such measures, it is doubtful that mycotoxins can be totally eliminated from the diet. Such measures may reduce contamination to the lowest possible levels, thereby removing a large part of the problem. However, the real significance of the residual low levels of potent toxins and carcinogens in foods is still left in doubt. Moreover, these contaminants must be considered in the context of the much larger problem posed by the whole range of biologically active agents that find their way into foods from a variety of sources. The fact that some of these toxins generally occur at very low levels may often make their presence toxicologically insignificant. However, the same generalization cannot be applied to contaminants with carcinogenic or mutagenic properties, because ineffective levels of such substances cannot presently be estimated in view of their irreversible effects on the cellular genetic apparatus.

Assessment of human risk from mycotoxins therefore is necessarily based largely on data from animals and other biological assay systems and are subject to all of the attendant uncertainties inherent in such extrapolations. A more complete understanding of the cellular processes affected by the toxins and appropriate metabolic studies with human tissues coupled with epidemiologic data may ultimately provide the basis for a more realistic assessment of the hazards from mycotoxins.

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