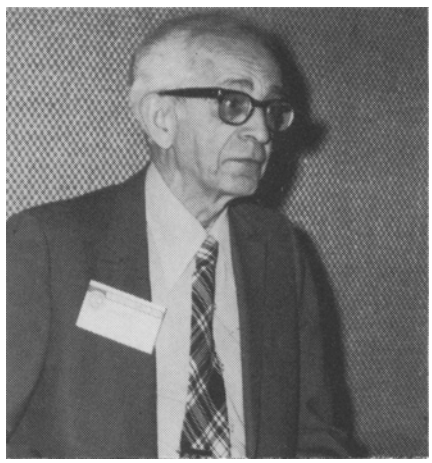
Mycotoxins – Past, Present and Future....

Leo A. Goldblatt – Bailey Award Winner



Dr. Leo Goldblatt speaks to North Central Section

I would like first to express my appreciation to the North Central Section for this award. I value it partly because of the caliber of the recipients who preceded me, partly also because I had the privilege of working in the same laboratory with Dr. Bailey in New Orleans for several years in the early 1940s. I am grateful to the Section, to the Awards Committee, and to the nominators.

My topic tonight, and I should credit the title to Dr. Krishnamurthy, is "Mycotoxins–Past, Present, and Future." I shall cover each topic but emphasize just a few areas, especially as they concern aflatoxin, still the most important mycotoxin.

Mycotoxins may be defined as toxic compounds produced by fungal contamination of foods and feeds. The toxicity syndromes resulting from the intake of such contaminated material by animals and man, usually by ingestion, have been termed mycotoxicoses to distinguish them from mycoses, which involve a generalized invasion of living tissue by actively growing molds. A mycotoxin is not itself a mold; it is a toxic material produced by a mold. The mold is a living organism; a mycotoxin is not.

Toxic factors of mold origin form a branch of study that has only recently aroused much interest. People born more than about fifty years ago were probably reared in the tradition that treated molds as a regrettable nuisance that detracted from the appearance of foods or made them taste bad and caused some loss or spoilage. Interest in mold-induced damage to foods and feeds centered primarily on economic losses. In fact, an eminent professor of bacteriology concluded in 1944 that, "There is very little evidence that moldy food causes illnesses." Then, 30 to 35 years ago, studies on penicillin and other antibiotics showed that some mold metabolites were toxic to pathogenic bacteria and could save people from hitherto fatal infections. Because of the tremendous surge of interest, people brought up in the 1940s and 1950s may have accepted the view that molds and mold metabolites are friendly things that help us to counteract dangerous infections. The fact that many of these mold metabolites could not be used because they were too dangerous for the host was obscured by the number and variety of the new wonder antibiotics. That these mold metabolites were effective only because they were poisonous to some other living things (bacteria) was also obscured. Penicillin is a mycotoxin for bacteria and lower forms of life, but not for man and most higher forms of life. Recognition in the early 1960s that some mold metabolites could be very poisonous to higher forms of life apparently came as an unpleasant surprise.

Mycotoxicoses (illnesses produced by mycotoxins) have been known for a long time although perhaps not by that name. Probably the first mycotoxicosis to have been recognized was ergotism. Ergotism has its origin in ingestion of rye and other grains infested with the mold *Claviceps purpurea*. During feudal days in Europe, periodic outbreaks of the disease, known as "St. Anthony's Fire" or the "Holy Fire," caused thousands of deaths. Epidemics of ergotism are now rare, but outbreaks occurred in Russia in 1926-1927, in England in 1928, and in France as recently as 1951 when hundreds of people were affected and a number died. Occasional isolated episodes are still reported. Even though this disease is old and well known, and its cause and prevention are also well known, the need for continual vigilance is evident. Also, although the hazard to the human food supply has largely been eliminated, contamination of certain types of pasture grasses continues to cause problems and is economically significant to livestock producers in some areas. One recent report said that the disease is increasing in the western United States because of open-pollinated and susceptible varieties of wheat. Despite the fact that we have had the problem of ergotism with us for so long, it has not been solved. All that we can say even now is that it is being controlled.

One more thing to bring you up to date on ergotism. Last year there were two articles about ergotism in *Science*, the weekly publication of the AAAS. In April, in a six-page article, the author suggested that the 20 residents of Salem, MA, who were executed for witchcraft in 1692 may have died because their accusers were suffering from ergot poisoning. One component of the toxins in ergot is the amide of lysergic acid, a close relative of the hallucinogen, LSD. LSD is the diethyl amide of lysergic acid. The author suggested that the accusers may have had a bad trip from eating grain contaminated with ergot and provided a good deal of convincing circumstantial evidence. Then in the December 24, 1976 issue of *Science* there was another five- or six-page article in which the authors take issue with the author of the first article and demolished all its arguments.

Another mycotoxicosis that has seriously affected human populations directly is alimentary toxic aleukia, ATA. The disease has been recorded in Russia from time to time at least as far back as the nineteenth century. Concurrent outbreaks in domestic animals have been reported also. The disease occurred with special severity during the war and postwar years of 1942-47, and Joffe reported that in 1944, the peak year, more than 10% of the population of certain districts was affected and thousands of people died. The disorder develops after eating overwintered moldy grain, chiefly millet, but also wheat, rye, oats, and buckwheat. Extensive studies by Russian investigators indicated that the fungi primarily responsible belong to the genera *Fusarium* and *Cladosporium*, which are highly resistant to cold and require a low temperature to produce the toxin. Actually, they do best in a refrigerator at about 0 C. Obviously, low temperature does not necessarily prevent elaboration of mycotoxins.

from St. Anthony's Fire to Salem's witches and a very dry martini

Persons at the Bailey Award dinner head table included, left to right, Lars Weidermann, Swift & Co.; Bernie Szuhaj, Central Soya; Tom Applewhite, Harriet Applewhite, Kraft; Dr. Leo Goldblatt; Bob Husch, Interstate Foods.

It has been reported that ATA is one of the most important human mycotoxicoses known. Nevertheless, as recently as 1962, Forgacs aptly called the mycotoxicoses "the neglected diseases" and wrote, "Of the innumerable diseases that affect man and domestic animals, the mycotoxicoses are perhaps the most unfamiliar and least investigated." In that same year, in a comprehensive review of more than 100 pages devoted mainly to veterinary mycotoxicoses, Forgacs and Carll noted that it is well recognized that fungi are among the most potent producers of biologically active organic compounds of great variety and wide spectra. However, they observed, "Scientists tend to approach the causes of animal diseases through a process of elimination; if the causal agent is not found to be bacterial, viral, or nutritional, it is concluded to be chemical in nature. Even though this is true, the possibility that the source of such toxic chemicals may be *fungal* in origin, is usually ignored." But this situation changed drastically when an apparently new disease appeared in England in 1960 and was termed the "Turkey-X Disease." Reports of the deaths in the course of a few months of more than 100,000 young turkeys on poultry farms in England dramatized the problem. Nor was the difficulty limited to turkeys–deaths of thousands of ducklings and young pheasants on nearby farms were reported. Reports of thousands of mortalities focused attention upon the practial problem and stimulated the interest of the scientific community.

Six characteristics of mycotoxin situations have been delineated by Feuell. These are:

(a) They frequently arise as veterinary problems whose true cause is not readily recognized.
(b) The disorders are not transmissible from one animal to another being neither infectious nor contagious.
(c) Treatment with drugs or antibiotics usually has little effect on the course of the disease.
(d) In field outbreaks the trouble is often seasonal, as particular climatic sequences may favor toxin production by the mold.
(e) Careful study indicates association with a specific foodstuff, e.g., peanut meal, rice, or corn.
(f) Examination of the suspected foodstuff reveals signs of fungal activity.

Such criteria are equally applicable to disorders in both animal and man. It is noteworthy how closely the Turkey-X disease problem exemplified the characteristic features of mycotoxin problems in general. And it is interesting, in retrospect, to see how closely efforts to solve the Turkey-X disease problem followed the pattern outlined by Forgacs and Carll.

Veterinarians and scientists of all kinds–pathologists, microbiologists, nutritionists, chemists, and others–were called in to seek a solution. Everything they found in an intensive investigation was negative. It was not any of about 50 different known chemical poisons they tested for. It was not a plant poison such as an alkaloid. It was not a lot of other things they looked for. They could not find any virus or bacterial infection or any new microorganism. Eventually the toxicity was traced to the feed, and more specifically to one ingredient in the feed, a peanut meal that had been imported from Brazil. It was given the name Rossetti meal from the name of the ship that brought thousands of tons of it to England.

During this time a test for the toxin was developed using young ducklings. These were found to be particularly susceptible and especially suitable for evaluation of toxicity based upon death or the appearance of characteristic liver lesions.

The test was used, too, to effectively monitor the extraction and concentration of the toxin by classical chemical procedures. Toxic extracts emitted a characteristic bright blue fluorescence when illuminated with ultraviolet light. Further, the intensity of fluorescence, as estimated visually, generally afforded a convenient guide to the toxicity of the sample. Thus was provided for the first time the basis for routine chemical assay of the toxin. About this time, too, it was recognized that the toxin is produced by the very common mold *Aspergillus flavus.* So the name Aflatoxin was coined from *A*spergillus *Fla*vus *Toxin.*

With the availability of a simple chemical means for detection and assay of the toxin and means for conveniently producing relatively large amounts, a number of laboratories undertook its preparation. Evidence was soon adduced by several groups that the toxin, even after partial purification, was a complex mixture affording up to 20 fluorescent spots on thin layer chromatography (TLC). Two major components, one fluorescing blue and the other green when exposed to ultraviolet light, were designated aflatoxins B and G. But it was soon recognized that each of these comprised two components, differing somewhat in R_f on thin layer chromatography. The four components were designated aflatoxins B_1, B_2, G_1, and G_2 in order of decreasing R_f value. About this time, late in 1961, came the first report of carcinogenicity. The report concluded: "After six months' feeding of 20 percent [toxic] Brazilian groundnut meal in a purified diet, nine out of eleven rats developed multiple liver tumors, and two of these had lung metastases. This finding indicates that this diet is carcinogenic." That report really started things moving.

Now we have an interesting coincidence. In April 1960 (the Turkey-X disease occurred in England in 1960) a shipment of live rainbow trout from a commercial fish hatchery in Idaho was stopped at the California border for a routine entrance inspection. It was then found that many of the trout had hepatomas (liver cancer). In 1963 the cause was eventually attributed to aflatoxin, but this time it was presumably due to a cottonseed meal, contaminated with aflatoxin, in the ration of the hatchery trout. By early in 1963, workers at Auburn University in Alabama reported that rats that had been fed domestic peanut meal developed tumors, again due to aflatoxin. So we knew we had an

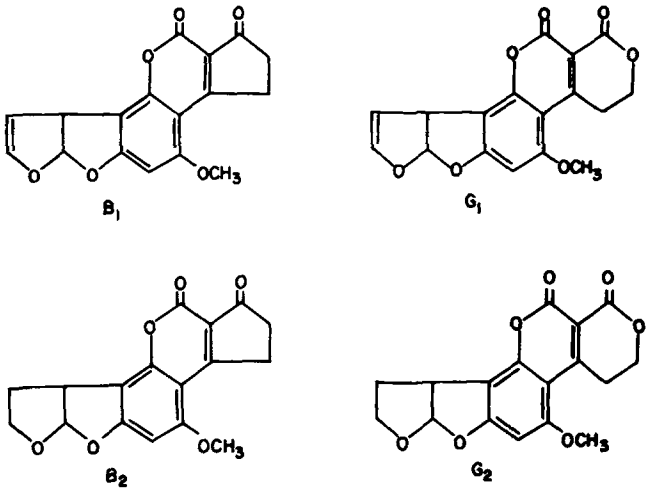


FIG. 1. Structures of aflatoxins B_1, B_2, G_1, and G_2.

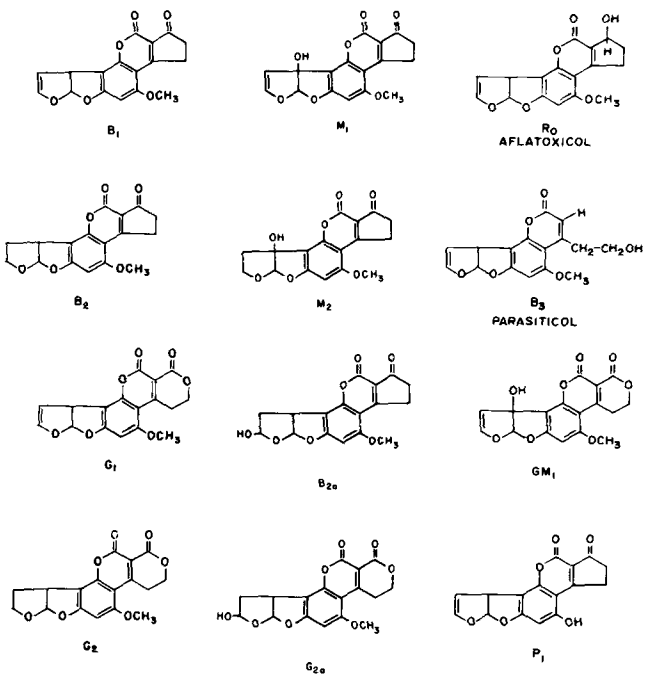


FIG. 2. Structures of 12 aflatoxins.

aflatoxin problem with both peanuts and cottonseed in the U.S., too. It wasn't just something that could happen in an undeveloped country like Brazil.

Also in 1963, it was reported that cows ingesting aflatoxin-containing rations excreted in the milk a toxic factor having a biological effect in ducklings similar to that caused by aflatoxin, but which was not any of the known aflatoxins. Later it was shown that the toxic material was composed of two closely related toxins; these were named aflatoxin M_1 and M_2, the milk toxins.

By this time, 1963, the structures of some of the aflatoxins were being determined. The structures of the first four aflatoxins, B_1, B_2, G_1, and G_2, are shown in Figure 1. The establishment of these structures so soon after their discovery—and with so little material to work with, actually only a few milligrams—is one of the notable accomplishments in the chemistry of natural products in recent times. I would call especially to your attention the great similarity of their chemical structures. The only difference between B_1 and B_2 and between G_1 and G_2 is the presence of the isolated double bond in the lower left corner of the structures of B_1 and G_1. The only difference between the B's and G's is the extra oxygen atom in the upper right corner of the G's.

In later years, additional products were discovered, and more than a dozen aflatoxins are now generally recognized. The structures of 12 of them are shown in Figure 2. The milk toxins M_1 and M_2 in the second column are hydroxylated B_1 and G_1. The first four do not have a free hydroxyl group. In the second column are also shown B_{2a} and G_{2a}. These are hydroxylated B_2 and G_2, formed by the hydration of B_1 and G_1, but the hydroxyl groups are in a different position than in the milk toxins. These four are all hydroxylated. That was the situation a few years ago with aflatoxin P_1, first reported in 1971 as the major metabolite of aflatoxin B_1 in monkey urine. It is the only phenol in these twelve aflatoxins and, essentially, CH_2 has been removed from the OCH_3 group.

Several more aflatoxins have since been reported. Aflatoxin D_1 is a major product obtained when B_1 is detoxified with ammonia under pressure; that will be discussed in more detail later. Aflatoxin Q_1 is the major product of in vitro conversion of aflatoxin B_1 by monkey liver tissue and accounts for up to 55 percent of the B_1 converted. About 2 to 3 percent is also converted to M_1 at the same time. Aflatoxin Q_1 is an isomer of M_1, but the hydroxyl is on the carbon atom beta to the carbon of the cyclopentenone ring. Aflatoxicol H_1 is another major in vitro conversion product of aflatoxin B_1 in human and monkey liver tissue. Aflatoxicol is shown in the upper right corner of this figure, and at one time it was called aflatoxin R_0. It is aflatoxin B_1 in which the carbonyl of the cyclopentenone has been reduced to the alcohol. Aflatoxicol H_1 is aflatoxin Q_1 in which the carbonyl has been reduced as in aflatoxicol. Essentially, the carbonyl has been reduced and the beta carbon oxidized.

Much effort has been devoted to determining the biological effects of the aflatoxins on various plant and animal species, to ascertaining "no-effect" levels of aflatoxins, and to determining their metabolic fate. All of the aflatoxins are very closely related chemically, but they may differ greatly in their biological effects. The toxic properties manifest themselves differently depending upon the test system, the dose, and the duration of exposure. In all species studied, sensitivity decreases with age; the young are most sensitive. Actually, the suffix "toxin" may be a misnomer in some cases because some of the aflatoxins would probably be considered nontoxic. The young duckling is one of the most sensitive species, but feeding young ducklings aflatoxin B_{2a} in an amount 50 to 100 times the amount of B_1 that would kill them appears to have no adverse effect. So we have a difference of at least 100-fold in the toxicity of these two very closely related toxins. It isn't just the difference of a hydroxyl group because aflatoxin M_1 appears to be very nearly as toxic as aflatoxin B_1.

Some aflatoxins are highly carcinogenic for some animal species. Again, aflatoxin B_1, the one that is most toxic, is also the most carcinogenic. The rainbow trout appears to be the most sensitive in this respect, and hepatomas develop at dietary levels of about 1 ppb of aflatoxin B_1, or even less. That should easily qualify aflatoxin B_1 as the most potent known carcinogen for rainbow trout, but closely related species of trout are far more resistant. Aflatoxin M_1 is also a potent carcinogen to rainbow trout. Sensitive strains of rats will also develop tumors at levels of 1 part per billion aflatoxin B_1 in the ration. And that is an extremely low level that is very hard to comprehend. My way of visualizing it is this: If you add one jigger of vermouth to a standard tank car full of gin, you would have a very dry martini with about 1 part per million of vermouth; divide that one jigger among a *thousand* tank cars and you would have about one part per billion.

Aflatoxins have other genetic effects also. Aflatoxin B_1 is mutagenic and teratogenic as well as carcinogenic. But

still another dimension was added when it was reported that there is an interaction between aflatoxins and cyclopropenoid fatty acids fed to rainbow trout. Certain cyclopropenoid fatty acids—malvalic and sterculic—are constituents of the lipids of cottonseed and the seeds of other plants of the order Malvales. Cottonseed meal, which contains some residual oil, was involved in the original 1963 report of tumors in hatchery trout. Addition of cyclopropenoids to the diet of rainbow trout promoted early development of tumors and increased their incidence and growth rate over positive controls. Also, it has been shown that two mycotoxins may have synergistic effects in host animals. A synergistic effect on tumor incidence in trout has been demonstrated between aflatoxins B_1 and B_2, and on toxicity symptoms in rats, not attributable to either compound alone, between aflatoxin B_1 and another mycotoxin, rubratoxin B.

A few words about Analytical Methodology. I want to emphasize the importance of adequate analytical methodology because it is an aspect that is so often neglected until it becomes abundantly clear that it is essential for further progress. The first analytical test for aflatoxin was its effect on young ducklings and the appearance of characteristic liver lesions. Then a chemical test was developed, based on the fluorescence of toxic extracts when illuminated with ultraviolet light. That, essentially, is the basis for all of the methods that are used now for the determination of aflatoxins. The steps that are used include preparation of the sample, extraction with a suitable solvent, concentration of the extract, partial purification, separation by thin layer chromatography, and finally comparison of the intensity of fluorescence with a standard, visually or with a densitometer. Figure 3 shows the kind of spots one sees on a thin layer plate when different quantities of a standard solution of aflatoxins B_1, B_2, G_1, and G_2 are spotted on a plate and developed. In an analysis, the spots obtained from a known quantity of an extract of the sample may be compared visually with the standard spots. Of course, that visual comparison of intensity of fluorescence is pretty subjective. Or, the intensity can be measured objectively with a densitometer. Figure 4 shows the kind of curve one gets when the intensity of fluorescence of a standard is measured with a densitometer. Note the excellent resolution of the four aflatoxins and the return to the base line.

Recently, there has been much interest in the use of high pressure liquid chromatography (HPLC) for the determination of aflatoxin. That skips the TLC step, and aflatoxin is determined either by ultraviolet absorption or by fluorimetry. From the data in several publications I have seen recently, it appears that much better reproducibility can be obtained by HPLC than by TLC.

No collaborative studies of the use of HPLC for determining aflatoxin have been made so official methods are not yet available, but such studies will probably be under way very soon. Also, I was interested to see recently an article that reported detection of aflatoxin B_1, in the parts per TRILLION range, using HPLC and fluorimetry in ethanol. The reference is *JACS* 98:620-621 (1976).

At first, the elapsed time for a single chemical analysis for aflatoxin was nearly three days. Since then, methodology has been improved to increase sensitivity, accuracy, reproducibility, and objectivity, and to decrease time. Now, in only a few hours, many commodities can be assayed for several aflatoxins, with a sensitivity of less than 1 ppb (1 μg/kg).

There is still no single quantitative analytical method suitable for the determination of aflatoxin in all commodities. Thus, a method that is quite satisfactory for peanuts is not suitable for cottonseed products and that, in turn, is not suitable for eggs, for alfalfa, or for mixed animal feeds.

In the U.S. today, we have methods officially recognized

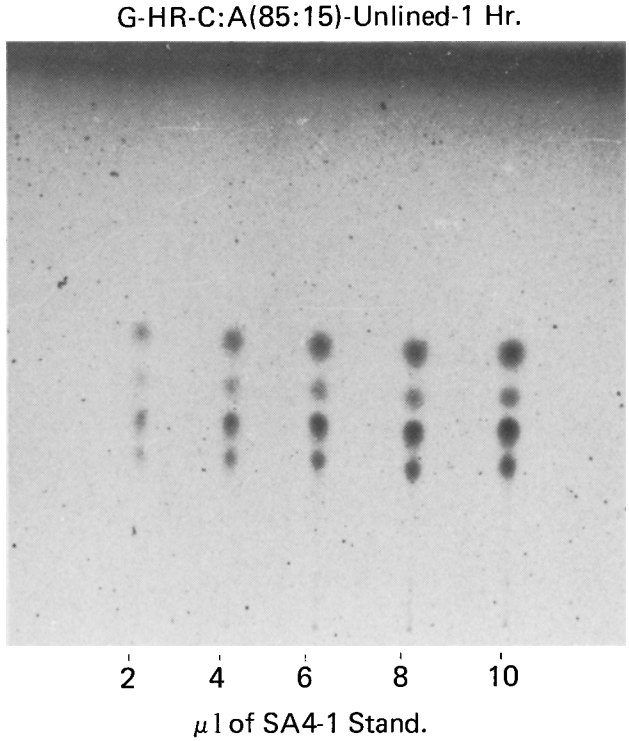


FIG. 3. Thin layer chromatogram of aflatoxins B_1, B_2, G_1, and G_2.

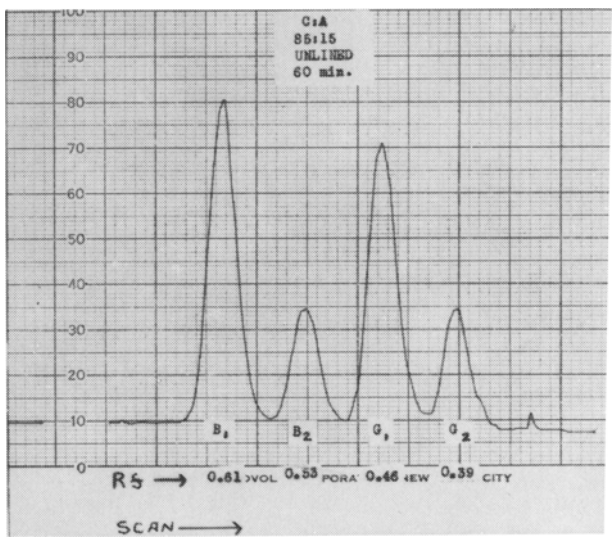


FIG. 4. Recorder trace of aflatoxins B_1, B_2, G_1, and G_2 on TLC plate.

by such organizations as the Association of Official Analytical Chemists (AOAC), the American Oil Chemists' Society (AOCS), and the American Association of Cereal Chemists (AACC) for the determination of aflatoxin in such products as peanuts, cottonseed, green coffee, and corn. An index to the mycotoxins of interest in the U.S. is the list of those that are selected by the AOAC for development of analytical methodology. In addition to associate referees appointed for development of analytical methods for aflatoxins, associate referees have been appointed for only seven other mycotoxins. Those are citrinin, ochratoxins, patulin, penicillic acid, sterigmatocystin, tricothecenes, and zearalenone.

What can be said about the occurrence and control or prevention of aflatoxin? Many millions of dollars have been spent on research on aflatoxin and literally more than two thousand scientific papers dealing with it have been

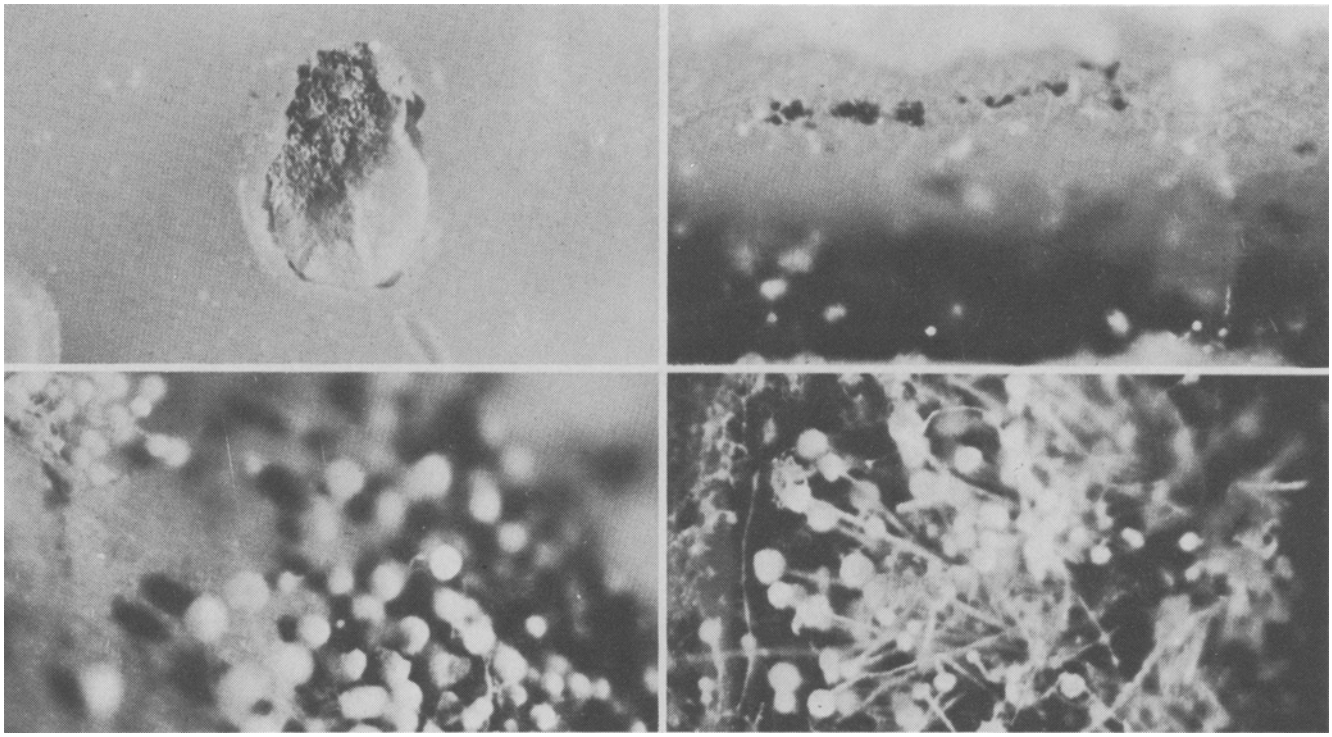


FIG. 5. *Aspergillus flavus* mold.

published. We now know much more about aflatoxin than we did ten years ago, but we still do not have the complete answer. As recently as five years ago, most people considered aflatoxin as primarily a storage problem. About that time a prominent scientist wrote: "There would be no aflatoxin problem if simple preventive measures were universally practiced." *Aspergillus flavus* was considered a storage mold, and it was believed that problems arose primarily, if not exclusively, after harvesting. We know now that although it can be a storage problem, it is also a field problem. We know that aflatoxin can frequently be found both in peanuts in the ground and in freshly dug peanuts. We know it can be found in corn growing in the field and at least in some tree nuts while the nuts are on the tree. In the case of cottonseed, it appears now that the problem is pretty much confined to a few locations and that it is primarily a field problem, exactly the reverse of what we thought just a few years ago.

The first step for control is recognition and awareness that the threat exists. Motivation of untrained personnel at all stages of culture, harvest, transportation, storage and processing is of vital importance. The Agricultural Research Service of the USDA has issued a special report, ARS 20-16, entitled "Preventing Mold-Caused Toxins in Farm Commodities," which should be very helpful. This report emphasizes the importance of good farm management practices and notes that mold prevention should begin with proper planting and growing of the crop and that it is the farmer's responsibility to take the proper measures to prevent mold damage to commodities before they reach marketing channels.

The same report also notes that special attention should be given to detecting lots that contain aflatoxin and diverting them from food and feed channels as early as possible in the marketing process. Early detection and diversion of small consignments of contaminated materials may prevent contamination of much larger supplies. To achieve this, rapid screening methods of detection are required and they are becoming available. Until fairly recently, primary emphasis on improving analytical methodology for aflatoxins was to increase sensitivity and accuracy, with relatively little emphasis on decreased time, especially if it meant a sacrifice in sensitivity, accuracy, or reproducibility. Lately, however, it has been recognized that a relatively insensitive method can be extremely useful if it is also very rapid.

It was noted several years ago that there is a high correlation between the aflatoxin content of peanuts and presence of *Aspergillus flavus* mold that is readily observable in damaged peanut kernels viewed under low magnification. That is, if *A. flavus* mold can be seen in any of the damaged kernels in a lot, there is a strong probability that a high level of aflatoxin will be found in that lot of peanuts. Conversely, if no *A. flavus* mold can be seen in the damaged kernels, there is only a slight probability of finding aflatoxin in that lot. And even if aflatoxin is found, where there is no observable *A. flavus* mold in the damaged kernels, the level of aflatoxin present is usually very much lower. The commercial grading system for peanuts in the United States—and the grade is the basis for the price paid to the farmer—calls for an actual count of the number of damaged kernels of peanuts in the grading sample. Another step was therefore introduced into the inspection system—examination of the damaged kernels for *A. flavus* mold. Each inspector is given a folder with two sets of colored photographs, about 20 by 30 cm, that show him what to look for and what not to look for.

Figure 5 shows what they are told to look for—*A. flavus* mold at different stages of development. Figure 6 shows characteristics of other types of molds that they might find but that they should not call *A. flavus*. For instance, at the bottom of the figure are the black balls (conidial heads) that are characteristic of *A. Niger*, which does not produce aflatoxin. This additional inspection operation takes less than a minute for each sample, but it has been extremely valuable to the peanut industry in the U.S. by diverting contaminated lots of peanuts from the food supply chain. There is no comparable test for cottonseed, but a simple and rapid procedure was recently developed to detect aflatoxin in cottonseed (aflatoxin itself, not the mold). The test has been called the millicolumn or minicolumn test.

A millicolumn of the type that is used is shown in Figure 7. It is a small tube about 4 mm ID and about 20 cm long, packed to a height of 1.5 cm with acidic alumina at the

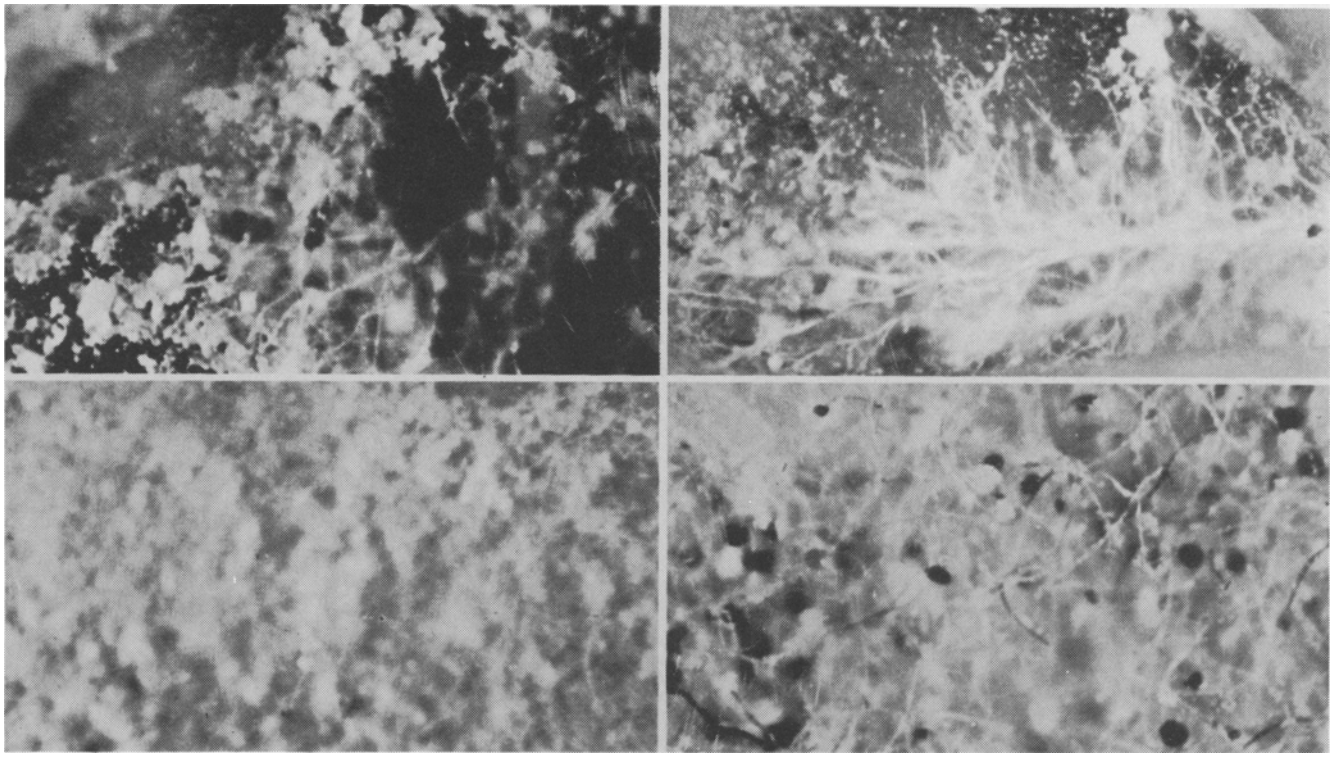


FIG. 6. Characteristics of types of molds other than *A. flavus*.

bottom and for 9 cm with silica gel. These are kept in place with small cotton plugs. The millicolumn test is sensitive to about 5-10 ppb and can be completed in 15 minutes or even less. Figure 8 shows the results obtained with yellow corn and yellow cornmeal spiked at 0, 20, 50, and 100 ppb. The arrows point to where the aflatoxin should be. The test is semiquantitative in that differences between 10, 50, and 100 ppb could probably be distinguished without much difficulty but not between 15 and 20 ppb or between 50, 60, and 70 ppb. This procedure has been used on a large scale in recent years to detect lots of contaminated cottonseed before they are unloaded from trucks; the cottonseed is then diverted for separate processing. The procedure has also been used with other commodities, including peanuts, corn, and tree nuts such as pecans and pistachio nuts, and it has been tested successfully with various other oilseeds and grains. Such procedures are useful for segregation of contaminated lots of materials and for control during processing. Also, this test is much less expensive than the usual quantitative methods, no small matter in view of the large number of tests that must be made for adequate control.

Advances in basic knowledge and in techniques for growing, harvesting, and handling crops will make it easier to produce and market commodities free of mold damage. Unquestionably the best approach is prevention. In some cases, antifungal agents may be helpful. Quite large amounts of a mixture of acetic and propionic acid are sometimes used to protect wet corn. But contamination with aflatoxin can occur despite the most strenuous efforts at prevention and even in the field before harvest. So detoxification must be considered, fully recognizing that it should be applied only if preventive measures have failed, not as an alternative to good cultural and storage practice.

The detoxification of commodities contaminated with aflatoxin will not be discussed in detail. Many approaches have been investigated. Successful procedures range from removal of contaminated material by hand and by electronic or pneumatic sorting to extraction with solvents or various chemical treatments. Each process has merit in specific applications and is currently is use; for example, in the mechanical sorting of peanuts and Brazil nuts, in the

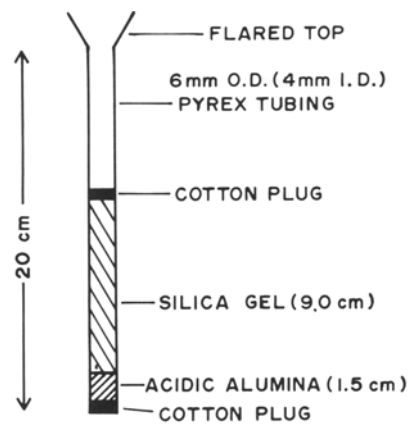


FIG. 7. Schematic of a millicolumn.

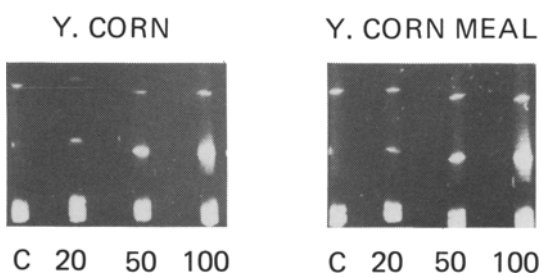


FIG. 8. Millicolumn showing results with extracts of spiked corn and corn meal.

removal of aflatoxins from crude oils by refining with aqueous alkali, and in the destruction of aflatoxin in cottonseed meal by ammonia under heat and pressure. Presumably each commodity, each mycotoxin, and perhaps each location has its special problems and solutions.

The only commercial chemical detoxification now employed that I am aware of is the destruction of aflatoxin in cottonseed and cottonseed meal by ammoniation. The

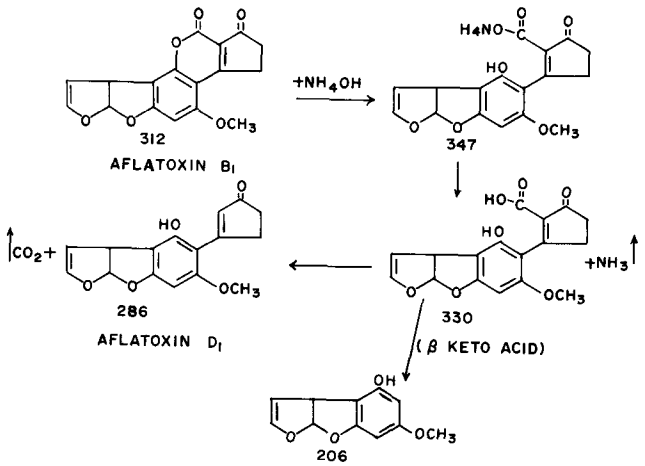


FIG. 9. A proposed scheme for the ammoniation of aflatoxin B_1 to produce aflatoxin D_1 and molecular weight 206 compound.

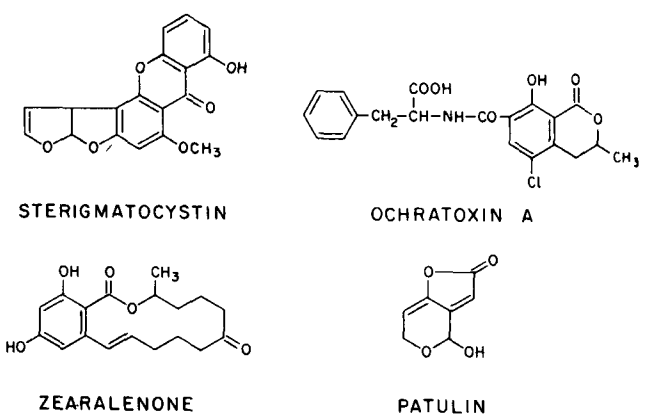


FIG. 10. Structures of sterigmatocystin, ochratoxin A, zearalenone, and patulin.

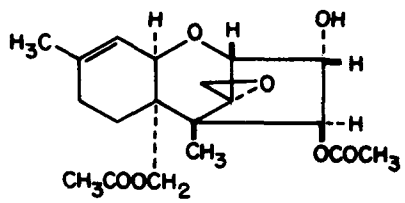


FIG. 11. Structure of diacetoxyscirpenol.

process for cottonseed meal developed on a batch scale in New Orleans involves heating moist cottonseed meal with ammonia under a pressure of about 40 lb/sq in. for about one-half hour at about 200 F. Results of preliminary short- and long-term feeding tests with ammonia-treated cottonseed meal on rats, poultry, rainbow trout, and beef and dairy cattle were the basis of limited approval by the FDA for ammoniated, aflatoxin-contaminated cottonseed meal, under certain restrictions of use and labeling. There are three installations in the U.S. to use this basic process. In one plant in the Los Angeles area, contaminated seed is detoxified with ammonia and the full fat ammoniated seed is marketed to the dairy industry. In Arizona, two plants treat contaminated cottonseed meal which may then be used for cattle and poultry.

Figure 9 shows what happens to aflatoxin B_1 when it is ammoniated under the conditions outlined—at least for the major reaction products. Ammonia reacts with aflatoxin B_1 at the lactone ring in the upper right to form an ammonium salt. This loses ammonia to produce the keto acid and that decomposes in two ways. One produces the compound molecular weight 286 that we have called aflatoxin D_1, for detoxified. The other produces a compound of molecular weight 206 which has been assigned the structure shown.

The need for motivating untrained personnel was mentioned earlier. An important factor here is simple unawareness. Despite Forgacs' characterization of the mycotoxicoses as the neglected diseases this fortunately is no longer the case, at least not by the scientific community as a few examples will indicate.

In 1970 in the U.S. there was an outbreak of Southern Corn Blight. That disease is incited by the fungal pathogen *Helminthosporium maydis*, and damage results when the fungus invades corn plant tissue in the field. According to a report in October 1970, "one of the first considerations when corn fields were invaded with Southern Corn Blight was whether the mold might form toxic compounds on corn harmful to man and animals." Contrast this with Forgacs' realistic appraisal of the situation before aflatoxin.

Another indication of the change is impressive. The frequency of multiple cases of deaths from cancer in the same house has given rise to the hypothesis of the existence of "cancer houses," which has been under discussion for many years. Recently there was a report of multiple cases of leukemia associated with one house; four cases occurred there over a ten-year period. Radiation surveys of the house and its surroundings revealed no abnormalities. It was concluded that the occurrence of multiple cases of leukemia in a single house is a rare event, that the significance of such events is hard to assess, but that their possible importance warrants close investigation. That report promptly led to a Letter to the Editor with the suggestion that a study of the mold population of the house be made. Awareness of the potential problem of toxicoses induced by molds is a valuable spinoff from aflatoxin research.

However, the awareness pertains to the scientific community. In 1970, the editor of the journal, *Food and Cosmetics Toxicology* wrote, "In the past ten years this toxin [aflatoxin] has given rise to an unprecedented torrent of publications from chemists, biochemists, mycologists, nutritionists, toxicologists and food technologists. No single compound or family of compounds has aroused so much concern in the field of environmental health." By 1970 there had been more than a thousand publications in the scientific and technical literature that dealt with aflatoxin, and the flow of publications on aflatoxin has not ceased. There are now well over two thousand publications dealing with aflatoxin, and publications on other mycotoxins are appearing at an increasing rate. But how much awareness is there on the part of the producer, the handler, the processor, and even management? The problem now may be not so much a lack of technical information as ineffective dissemination of existing knowledge. New approaches may have to be sought to educate appropriate personnel with the technical knowledge they should have.

What we have learned with aflatoxin may be very useful in dealing with other mycotoxin situations. What is the situation with respect to other mycotoxins? Although mycotoxins can be produced in the laboratory by a very large variety of molds on almost any agricultural commodity, conditions conducive to production of the mycotoxins may not prevail in nature, and the situations that have been known to induce disease in man or animal are more limited. So, although a great many toxic compounds produced by molds in the laboratory are known, the number known to have caused natural outbreaks of mycotoxicoses in farm animals or man is quite limited. A recent review by Dr. Fred Senti listed fewer than twenty. The principal fungal species involved in reported mycotoxicoses are the *Aspergilli, Fusaria,* and *Penicillia*. A major cause of concern with mycotoxins in foods is their possible carcinogenicity, partly perhaps because of the extreme carcinogenicity of some of the aflatoxins and partly because cancer can take such a long time to develop. Only a very few of

the known mycotoxins have been shown to be carcinogenic in any animal species. Actually, only sterigmatocystin, in addition to some of the aflatoxins, has been shown to be carcinogenic by feeding tests. Administered in a diet to rats, sterigmatocystin induces liver cancer. Two more mycotoxins, patulin and penicillic acid, have induced tumors in rats on subcutaneous injection but carcinogenicity in feeding tests has not been reported. And that's it for known carcinogenic mycotoxins that have been found in nature, at least in the U.S.

As I mentioned earlier, one indication of the mycotoxins that are of greatest interest in the U.S. is their selection by the AOAC for development of analytical methodology. As of now there are official methods for only four mycotoxins in addition to the aflatoxins. They are: ochratoxin, patulin, sterigmatocystin, and zearalenone. Associate referees have also been designated to develop methods for citrinin, penicillic acid, and tricothecenes which indicates an interest in them although analytical methodology suitable for official adoption has not yet been developed.

Of the known mycotoxins, those of most interest in the U.S. today, at least from a public health point of view, in addition to the aflatoxins, are citrinin, ochratoxins, patulin, penicillic acid, sterigmatocystin, tricothecenes, and zearalenone. The structures of the four mycotoxins for which we have official methods of analysis are shown in Figure 10.

Sterigmatocystin is structurally the most closely related to aflatoxin. Some consider it to be a precursor in the biosynthesis of aflatoxin. Yields as high as 1.2 g/kg (more than a million ppb) have been produced on corn meal. Although the carcinogenic potency is only about a tenth that of aflatoxin B_1, it was considered that sterigmatocystin could present an even greater hazard to human and animal health because in some cases it was produced in so much higher concentration. But no sterigmatocystin was detected in a survey of 457 samples of small grains in the U.S. by the FDA, or in 173 samples of various foods in South Africa. Last year the associate referee for sterigmatocystin reported, "No natural occurrence of sterigmatocystin in food or feed has yet been detected except in badly molded samples." He recommended that since present methods are adequate for survey purposes, until a sterigmatocystin contamination potential is demonstrated there be no further method development. The referee on mycotoxins concurred. Then, ironically, last September at the IUPAC meeting in Paris, the natural occurrence of sterigmatocystin in milk was reported!

Patulin is produced by a *Penicillium*, specifically *P. expansum* commonly found in decayed fruit. In the early 1940s, it was proposed as an antibiotic and was said to hold promise of therapeutic activity against the common cold. Later it was found almost useless for that and too toxic as an antimicrobial agent. Patulin is a mild antibiotic, and at an intermediate stage of a long-term test of orally administered patulin, the only acute effects observed can be related to its antibiotic effect on the gut microflora.

Patulin has been found in apples, apricots, grapes, pears, peaches, pineapples, and other fruits. It occurs commonly in apple juice and is related to inclusion of decayed apples with the fruit going to the presses. It has been reported that 9 of 40 samples of juice from 2 such mills sampled had patulin levels of 20-45 mg/liter. Alcoholic fermentation of a contaminated apple juice eliminates patulin. That could be comforting, but ethyl alcohol produced by yeasts has also been called a mycotoxin.

The strucutre of ochratoxin A is shown in Figure 10. Note the chlorine atom in the molecule. Molds can make chlorinated compounds, although that is not common. Three ochratoxins are known. In ochratoxin B, the chlorine is replaced by hydrogen. Ochratoxin C is the ethyl ester of ochratoxin A. Ochratoxins were first discovered in South Africa in laboratory cultures of *Aspergillus ochraceous* obtained from corn, but ochratoxin was first detected as a natural contaminant by workers at the Northern Regional Laboratory in Peoria in corn growing in the U.S. Also a *Penicillium, P. viridicatum,* seems generally to be a better producer of ochratoxin than *A. ochraceous*. Only ochratoxin A has been detected in most cases of natural occurrence; it has been incriminated in cases of kidney damage in swine, especially in Denmark, but it has also been reported in the U.S.

The last structure shown in this figure is zearalenone, produced by a species of *Fusarium. Fusaria* produce a number of toxins, and zearalenone is sometimes referred to as F-2 toxin. Some species of *Fusaria* produce significant quantities of F-2 toxin in corn. Consumption by swine in amounts of more than a few parts per million causes infertility in sows and economic loss from lowered production. There have been a number of outbreaks in the Midwest.

In Figure 11 is shown the structure of diacetoxyscirpenol. A group of related compounds produced by various species of *Fusaria*, but especially by *F. tricinctum*, are sometimes called scirpenes and sometimes tricothecenes. The structure is quite complex but more than 30 tricothecenes are now known and several naturally occurring tricothecenes have been implicated in economically important mycotoxicoses.

Fusarium species may be found in many kinds of plants and plant materials but the toxins are encountered in the U.S. almost exclusively in corn because of the conditions required for their production, especially the right moisture content and temperature. However, the toxins have been involved in a number of episodes in the Midwest. Diacetoxyscirpenol and T-2 toxin in cattle may produce various digestive disorders, massive hemorrhaging in various tissues, and often death. One tricothecene has been named vomitoxin because it induces prolonged vomiting in swine. It may be the so-called "refusal factor" that causes swine simply to refuse to eat corn contaminated with it. Circumstantial evidence points to the possible involvement of tricothecenes in ATA, the very important human mycotoxicosis that caused so many deaths in Russia in the 1940s.

Finally, where do we stand? Because of the aflatoxin problem there has been a tremendous surge of interest in mycotoxins among scientists of many disciplines. Mycotoxicoses are no longer the neglected diseases. Every known plant and animal product is susceptible to attack by molds under appropriate conditions, but the number of known mycotoxins of practical importance is relatively small. There now appears to be the same kind of scientific interest in mycotoxins as there was some 60 years ago in vitamins. At that time we began to be aware of illnesses caused by the absence from the diet of trace amounts of unrecognized materials that were called vitamins. Now we are dealing with illnesses caused by the presence in the diet of trace amounts of previously unrecognized materials that are called mycotoxins. Mycotoxicoses have been known for centuries, but we have succeeded only in controlling—not eradicating—even the best known mycotoxicosis, ergotism. Perfect safety may be the unattainable dream; we may have to learn to live with an acceptable level of risk. But with a new awareness, with continual surveillance, and perhaps thanks partly to aflatoxin, the consumer will receive better and safer products than ever before. ●