Toxins from Moldy Cereals

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Moldy cereals are frequently poisonous. Most known mycotoxins have been obtained from pure fungal cultures rather than from the contaminated cereals. Alimentary toxic aleukia (ATA) in Russia has been attributed to fusariogenin from *Fusarium poae* growing on overwintered millet and to epi- and fagicladosporic acids produced by Cladosporium species. Mold metabolites associated with yellow rice toxicosis in Japan include islanditoxin, patulin, luteoskyrin, citrinin, citreoviridin, and others, produced by various species of *Aspergillus* and *Penicillium*. Six ochratoxins are formed by *A. ochraceus* isolated from sorghum in South Africa. *P. cyclopium*, of world-wide occurrence on stored grains and cereal products, produces cyclopiazonic acid, an acutely toxic substance. Moldy corn has caused poisoning of poultry, swine, and cattle. Some of the fungi present and their toxic metabolites include the rubratoxins from *P. rubrum*, zearalenone from *F. graminarum*, and several trichothecanes, produced by *F. tricinctum*.

The title, "Toxins from Moldy Cereals," is oversimplified in that the toxic chemicals to be considered have not in general been obtained from the moldy cereals themselves, but from artificially grown pure cultures of fungi isolated from contaminated cereal grains or cereal products. The present review will be restricted to toxic metabolites of fungi that were isolated from moldy cereals, and hence will omit consideration of mycotoxicoses usually associated with other types of moldy products, such as straw, forage, peanuts, and the like. In particular, the aflatoxins will not be covered in detail because of the extensive literature already available on this group of compounds.

Some of the older, well-known mycotoxins will be mentioned briefly for completeness and relevant background, but chief emphasis will be given to results reported within the last several years. The chemical structures and LD_{50} values, where known, of many of the compounds to be considered, are given in Table I, approximately in the order in which they appear in the text. The orientation of this review is directed toward foods and feeds, and to the chemical nature and biological effects of mycotoxins that may be contained therein. Several other reviews on this subject have appeared within the last few years (Borker *et al.*, 1966; Brook and White, 1966; Ciegler and Lillehoj, 1968; Feuell, 1966; Forgacs and Carll, 1962; Mateles and Wogan, 1967; Petering, 1966; Wogan, 1965).

Alimentary Toxic Aleukia. The Soviet Union and Japan were the first countries to start organized research in the mycotoxin field. The impetus for this effort in the USSR came from the study of a recurring, widespread human disease first recognized as a problem in Russia in the early 1930's and now known as alimentary toxic aleukia (ATA). Extensive accounts of this disease and of the efforts made by Soviet workers to elucidate the causative factors are available (Forgacs and Carll, 1962; Joffe, 1962, 1965; Mayer, 1953). The disease is caused by consumption of moldy grain which has overwintered in the field. The molds primarily responsible appear to be various species of *Fusarium* and *Cladosporium*. The *Fusarium* species involved (*sporotrichioides, poae,* and *tricinctum*) have been termed synonomous by Snyder and Hansen (1945), who put them in *F. tricinctum*. The infected grains are chiefly millet, but also wheat, rye, oats, and buckwheat. Joffe (1965) emphasized that toxin production is greatest at low temperatures (about 0° to −10° C.).

The symptoms of ATA were described (Joffe, 1965) as "typical spots on the skin, leukopenia, agranulocytosis, necrotic angina, hemorrhagic diathesis, sepsis and exhaustion of the bone marrow." The clinical symptoms are similar to those that are produced by ionizing radiation or by benzene poisoning.

ATA must be listed as one of the most important human mycotoxicoses known, as large numbers of people in the agricultural areas of the Russian grainbelt (approximately 40° to 140° east longitude by 50° to 60° north latitude) have been poisoned, and death rates in some cases have run as high as 50 to 60% of those affected (Mayer, 1953). It is therefore a matter of great interest to identify the specific toxins responsible. Russian workers, guided by a rabbit skin irritation test, were able to isolate several toxic products from *Fusarium* and *Cladosporium* species. One, sporofusariogenin, obtained by Olifson (1959) is reported to be a steroidal unsaturated δ lactone. The structure (Table I) is reminiscent of the cardiac-active steroids (Fieser and Fieser, 1959). Compounds of this type are extremely poisonous, but act specifically on heart muscle and are not usually listed as skin irritants or compounds that attack the bone marrow. Two other toxins obtained from *Cladosporium* are reported to be long-chain unsaturated thiocarboxylic acids (Joffe, 1965; Olifson, 1960). The structures of these compounds, epicladosporic and fagicladosporic acids, are given in Table I. As far as the

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Table I. Structure and Toxicity of Mycotoxins from Fungi Associated with Cereal Grains

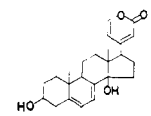
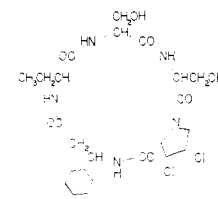
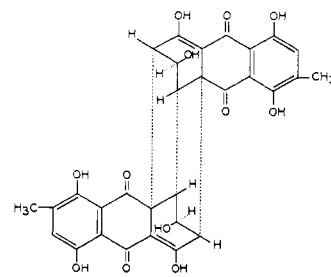
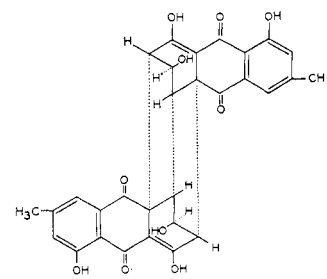
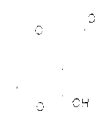
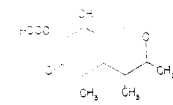
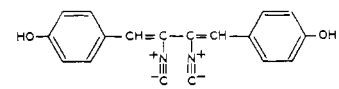
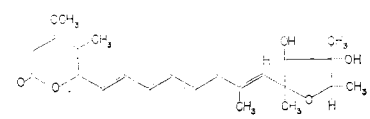
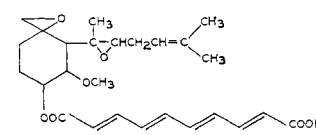
Mycotoxicosis and Fungi Involved	Mycotoxins Produced: Name and LD_{50}[a]	Structure	Reference
Alimentary toxic aleukia			
Fusarium tricinctum	Sporofusariogenin		Olifson, 1959
Cladosporium epiphylum	Epicladosporic acid	$CH_3(CH_2)_7CH{=}CH(CH_2)_{13}COSH$	Olifson, 1960
Cladosporium fagi	Fagicladosporic acid	$CH_3(CH_2)_9CH{=}CH(CH_2)_9COSH$	Olifson, 1960
Yellow rice disease			
Penicillium islandicum	Islanditoxin 0.3 mg./kg. iv. –1 0.47 mg./kg. sc. –1 6.5 mg./kg. oral –1		Marumo, 1955, 1959; Uraguchi *et al.*, 1961a
	Luteoskyrin 6.6 mg./kg. iv. –1 147 mg./kg. sc. –1 221 mg./kg. oral –1		Sankawa *et al.*, 1968; Uraguchi *et al.*, 1961a
P. rugulosum *P. tardum* *P. brenneum*	Rugulosin		Sankawa *et al.*, 1968
P. urticae	Patulin 10 mg./kg. sc. –1		Ugai *et al.*, 1954; Woodward and Singh, 1950
P. citrinum	Citrinin 35 mg./kg. sc. –1		Cartwright *et al.*, 1949
P. notatum	Xanthocillin		Hagedorn and Tönjes, 1957
P. citreoviride	Citreoviridin		Hirata, 1947; Sakabe *et al.*, 1964
Aspergillus fumigatus	Fumagillin 800 mg./kg. sc. –1		Tarbell *et al.*, 1962

Table I. (Continued)

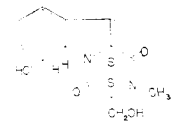
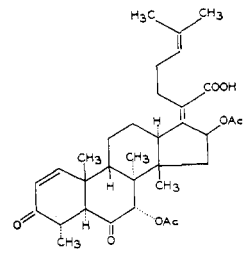
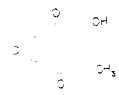
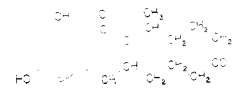
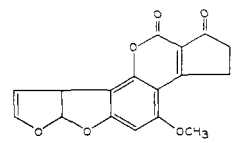
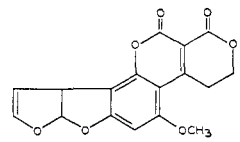
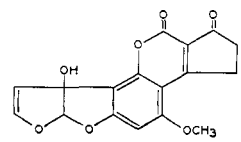
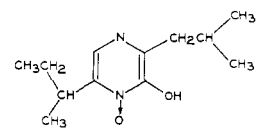
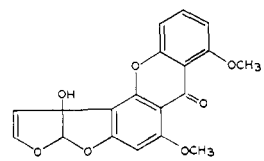
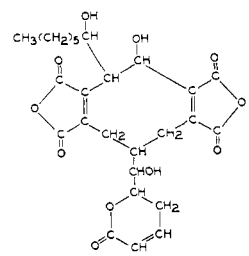
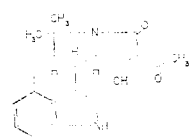
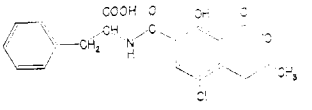
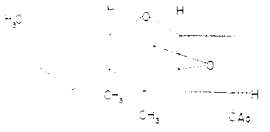
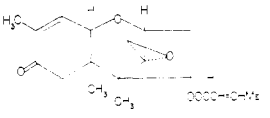
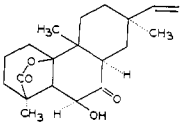
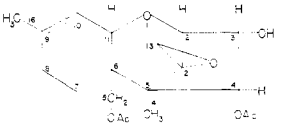
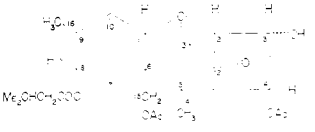
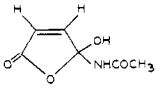
Mycotoxicosis and Fungi Involved	Mycotoxins Produced: Name and LD_{50}[a]	Structure	Reference
	Gliotoxin 45–65 mg./kg. sc. –1		Beecham, *et al.*, 1966
	Helvolic acid 400 mg./kg. ip. –1		Okuda *et al.*, 1967
A. terreus	Terreic acid 70–120 mg./kg. iv. –1		Sheehan *et al.*, 1958
Vulvo-vaginitis in swine *Fusarium graminearum* *Gibberella zeae*	Zearalenone		Urry *et al.*, 1966; Vlattas *et al.*, 1968
Moldy corn toxicosis *A. flavus*	Aflatoxin B_1 0.36 mg./kg. oral –3 5.5 mg./kg. oral –2		Asao *et al.*, 1965
	Aflatoxin G_1 0.78 mg./kg. oral –3		Asao *et al.*, 1965
	Aflatoxin M_1		Holzapfel *et al.*, 1966
	Aspergillic acid 150 mg./kg. ip. –1		Dutcher, 1958
	Aspertoxin		Rodricks, 1968; Rodricks *et al.*, 1968
Penicillium rubrum	Rubratoxin B 400 mg./kg. oral –1 2.6 mg./kg. ip. –1		Moss *et al.*, 1967, 1968
P. cyclopium	Cyclopiazonic acid		Holzapfel, 1968

Table I. (Continued)

Mycotoxicosis and Fungi Involved	Mycotoxins Produced: Name and LD_{50}[a]	Structure	Reference
Aspergillus ochraceus	Ochratoxin A 0.5 mg./kg. oral –3		Steyn and Holzapfel, 1967; Van Der Merwe *et al.*, 1965
Trichoderma viride	Trichodermin		Godtfredsen and Vangedal, 1965
Trichothecium roseum	Trichothecin 300 mg./kg. oral –1		Freeman, 1955; Godtfredsen and Vangedal, 1965
	6β-Hydroxy-rosenonolactone		Holzapfel and Steyn, 1968
Fusarium scirpi (*F. roseum*) *F. tricinctum*	Diacetoxyscirpenol 7.3 mg./kg. oral –1 0.75 mg./kg. ip. –1		Bamburg *et al.*, 1968b; Dawkins *et al.*, 1965; Flury *et al.*, 1965
Fusarium tricinctum	T-2 Toxin 4 mg./kg. oral –2		Bamburg *et al.*, 1968a, 1968b
	4-Acetamido-4-hydroxy-2-butenoic acid-γ-lactone 44 mg./kg. ip. –1		Yates *et al.*, 1968

[a] The number following the method of administration for the LD_{50} determination corresponds to one of the test animals listed below: 1, mice; 2, rats; 3, duckling.

authors are aware, such thioacids as a class are not highly toxic. The possible involvement of toxins of the trichothecane type in ATA is considered in connection with moldy corn toxicosis.

Yellow Rice Toxicosis. Stored rice containing more than 14 to 15% moisture is an ideal growth medium for many molds, particularly species of *Penicillium* and *Aspergillus*, the former of which make many red and yellow pigments and give the rice a yellow color. Climatic conditions in many Oriental countries make it difficult to avoid high moisture levels in grain.

The first important report on rice molds appeared in 1940, when Miyake *et al.* (1940) isolated *Penicillium citreoviride* from a rice sample that caused a fatal nervous disturbance. Citreoviridin, the toxic factor involved, was isolated by Hirata in 1947 and the structure was reported recently (Sakabe *et al.*, 1964). The lethal dose of citreoviridin in rats caused paralysis and death by affecting the motor ganglia. Sublethal doses caused swelling of the liver and kidneys, hemorrhage, and congestion of the nervous system (Wogan, 1965). Citreoviridin also produces cardiac and neurological symptoms in animals reminiscent of human beri-beri, and public health records show that the incidence of beri-beri in Japan can be correlated generally with the consumption of moldy, poor quality rice (Uraguchi, 1968). Thus, citreoviridin may be the "x factor" or "oryzaetoxin" long suspected to be involved in beri-beri (Wogan, 1965).

Another common mold, especially on polished rice, is *P. citrinum*, which produces a toxic metabolite, citrinin. This compound (Table I) causes noticeable lesions in the kidneys, especially affecting the renal tubules (Wogan, 1965). The LD_{50} of citrinin is about 35 mg. per kg. subcutaneously.

Probably the most toxic of the fungi from yellow rice is *Penicillium islandicum* (Uraguchi *et al.*, 1961b). Two toxic metabolites, islanditoxin, a chlorine-containing peptide, and luteoskyrin, a pigment, have been isolated and characterized (Table I) (Marumo, 1955, 1959; Uraguchi *et al.*, 1961a). Islanditoxin is a carcinogenic hepatotoxin with an oral LD_{50} in mice of 6.5 mg. per kg. Removal of the chlorine atoms, easily accomplished with 0.05*N* ammonia, gives a nontoxic product (Uraguchi *et al.*, 1961b). Luteoskyrin also causes extensive liver damage, particularly on a low-protein, rice-based

diet (Morooka *et al.*, 1966), but is much less toxic (oral LD_{50} in mice is 221 mg. per kg.) (Wogan, 1965). Feeding as little as 1% of the diet of moldy rice infected with *P. islandicum* induced liver cirrhosis in experimental animals (Wogan, 1965). Diffuse atrophy of the liver was seen in animals kept on a moldy rice–containing diet for long periods.

Many other toxin-producing species of *Penicillium* and *Aspergillus* are commonly isolated from rice. Pigments produced by many *Penicillium* species contribute to the yellow color of the rice and some, such as rugulosin and rubroskyrin, contribute also to its toxicity. The structures of these pigments and of luteoskyrin were revised in 1968 (Sankawa *et al.*, 1968) to those shown in Table I.

P. urticae, a known producer of patulin (structure given in Table I), is often found on moldy rice. Although there are no reports on poisoning in man due to this fungus, the mass death of cows that consumed malt feed infected with *P. urticae* has been reported (Hori *et al.*, 1954; Ugai *et al.*, 1954). There is evidence that patulin is carcinogenic (Dickens and Jones, 1961; Mayer and Legator, 1968). Several other molds also produce relatively large amounts of patulin, and at least two species occur on foods, viz., *Penicillium expansum*, the major cause of economic loss from the rotting of apples, and *Aspergillus clavitus*, toxic strains of which have been isolated from wheat flour.

A toxic metabolite of *Aspergillus fumigatus* called gliotoxin has recently been characterized (Beecham *et al.*, 1966). *A. fumigatus* also produces helvolic acid and fumagillin. The toxicity and structure of these metabolites are listed in Table I. Several other toxic metabolites of *Penicillium* and *Aspergillus* species which have been known for many years and probably contribute to moldy rice toxicosis are also listed in the table. The high incidence of liver diseases (cirrhosis, carcinoma, etc.) among rice-eating peoples, which probably is several hundred times higher than in the U.S., could well be due at least in part to *P. islandicum* metabolites. Other carcinogenic mycotoxins such as patulin and the aflatoxins may also be involved, but none of these substances have as yet been demonstrated to produce malignant tumors in man.

Vulvo-vaginitis in Swine. The first observation of an estrogenic effect from moldy feed was reported by McNutt at the University of Wisconsin in 1928 (McNutt *et al.*, 1928). The gilts that ingested this feed suffered from swollen vulvas and mammary glands. Sows consuming toxic feed aborted. In 1963, Stob *et al.* isolated an anabolic, uterotrophic compound from corn infected with *Gibberilla zeae*. Mirocha *et al.* (1967) later isolated the same compound, zearalenone, from stored corn infected with *Fusarium graminearum* (the imperfect stage of *G. zeae*). The structure of zearalenone was determined recently by Urry *et al.* (1966) (Table I), and a complete synthesis was accomplished (Vlattas *et al.*, 1968).

One milligram of zearalenone will cause a gilt to suffer swollen mammary glands and vulvo-vaginitis within 48 hours. At much lower concentrations, however, zearalenone is a good growth promoter in swine (Mirocha *et al.*, 1968a).

An analytical thin-layer chromatographic system has been developed to test for zearalenone in feeds (Eppley, 1967), and it may be analyzed also by gas-liquid chromatography (Mirocha *et al.*, 1968a). A whole series of compounds that have different R_f values from zearalenone, but which have similar fluorescent properties, has been discovered by Mirocha. Many of these compounds have similar biological activity, so that the problem in the field may be due to the whole complex. The isolation and characterization of these compounds are in progress (Mirocha *et al.*, 1968b).

Epidemic Polyuria. One of the most recently reported mycotoxicoses is called epidemic polyuria, a disease of humans caused by the consumption of millet grain infected with a strain of *Rhizopus nigricans* (Narasimhan *et al.*, 1967). The disease is characterized by polyuria, thirst, anorexia, weakness, and fatigue. Death has occurred in some cases, possibly due to electrolyte loss and imbalance. Work on the isolation of the mycotoxin and its possible relationship to the antidiuretic hormone are in progress (Narasimhan *et al.*, 1967).

Fungal Nephrotoxicity in Swine. Mold nephrosis, a kidney disease of swine caused by consumption of moldy feed, has been a problem for a long time, particularly in Denmark, where the incidence is estimated at about 3 to 7% of the animals marketed annually, with especially high frequency after wet harvesting periods (Krogh and Hasselager, 1968). Since the annual slaughter of pigs in Denmark is over 10 million, the number of animals affected may range up to 700,000 or more per year. Moldy rye and moldy barley have both been shown to cause the disease, and mycological examination of the grains revealed the presence of many species of *Penicillium* and *Aspergillus*. By growing pure cultures of the mold isolates on barley and testing their toxicity in rats, Krogh and Hasselager (1968) found that *P. viridicatum* was able to produce an orally active nephrotoxic principle and appeared to be the organism chiefly responsible for the practical problem in the field.

Purification of the toxin is in progress, but its chemical nature has not yet been reported. The symptoms produced in rats include greatly increased water consumption and urine output, depressed growth rate, and chronic kidney damage consisting of tubular degeneration and excessive formation of connective tissue. In swine under practical farm conditions, the difficulty is primarily growth retardation and development of enlarged, pale-colored kidneys. Similar symptoms are produced in rats by feeding the infected barley (Figure 1). The toxic molds obtained from the infected grain did not produce aflatoxins. Likewise, concentrates of the active principle gave no response in the rat skin irritation test.

Moldy Corn Toxicosis. Moldy corn toxicosis is one of the most complex of the mycotoxicoses, since in every outbreak of the disease different molds and different mycotoxins may be involved. There is also quite a great variation in response between animals to the same toxins; consequently, moldy corn toxicosis of pigs, poultry, and cattle are often discussed as separate diseases (Burnside *et al.*, 1957; Forgacs, 1966; Forgacs and Carll, 1962).

The major symptom of moldy corn toxicosis in both pigs and cattle is hemorrhaging in many tissues. Numerous petechial hemorrhages occur on the surface of the liver, stomach, esophagus, and other organs, while the

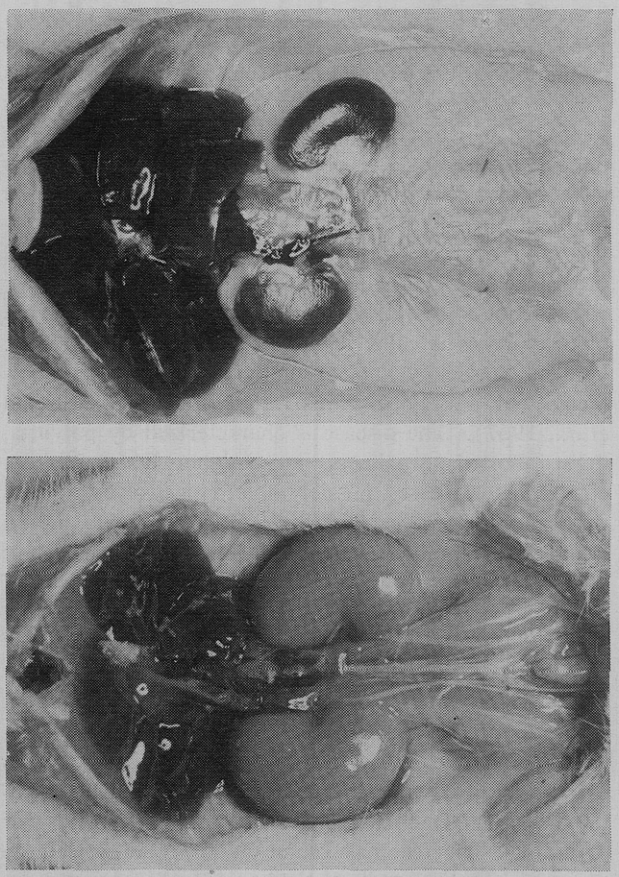


Figure 1. Upper—control rat fed normal barley for 32 days. Lower—rat fed a pure culture of *Penicillium viridicatum* on barley for 32 days. The organs except liver and kidneys have been removed

Magnification: 1.3 ×. Reproduced by permission from Krogh and Hesselager, Royal Veterinary and Agricultural College Yearbook, Copenhagen, Denmark, 1968

intestine and sometimes even the abdominal cavity of pigs are found filled with blood. Severe edema in the visceral organs of cattle is also typical (Wogan, 1965).

Toxic strains of many fungi have been isolated from moldy corn and several toxic metabolites have been isolated and characterized. *Aspergillus flavus* is one of the most commonly isolated fungi from stored feed. Several of the toxin-producing strains isolated produced aflatoxins, the most toxic group of mycotoxins known and, in very low doses, potent hepatocarcinogens (Wogan, 1966). Several other toxic metabolites of *A. flavus* are listed in Table I. Two of the more recently characterized are *O*-methylsterigmatocystin and aspertoxin (Rodricks, 1968; Rodricks *et al.*, 1968).

Penicillium rubrum produces a compound, rubratoxin B, which has been isolated and completely characterized (Moss *et al.*, 1967, 1968). It has an oral LD_{50} of 400 mg. per kg., hardly a toxin by some standards. In much lower doses than the LD_{50}, however, rubratoxin B has a synergistic action with aflatoxin and, since *A. flavus* and *P. rubrum* occur together frequently, rubratoxin B may play an important role in animal toxicity, though it is probably not carcinogenic (Büchi *et al.*, 1968).

Chaetomium globosum appears to produce a toxin on corn, although the affected corn was toxic only to rats and not to pigs. The toxin was isolated by Christensen *et al.* (1966).

A recently characterized mycotoxin from *Penicillium cyclopium* is cyclopiazonic acid (Holzapfel, 1968). Several strains of *P. cyclopium* were found to cause acute toxicosis in ducklings and rats, and cyclopiazonic acid was the causative agent. The compound causes a slight tremorgenic response in the animal before paralysis and death occur. This mold has a worldwide distribution and is often found on stored grain and cereal products (Holzapfel, 1968). It is also the major cause of decay in stored garlic (Smalley and Hansen, 1962), and therefore could possibly introduce cyclopiazonic acid into garlic-flavored food products.

A tremorgenic toxin, $C_{37}H_{44}O_6NCl$, was isolated from several strains of *P. cyclopium* recovered from moldy animal feeds (one mostly corn) and from peanuts (Wilson *et al.*, 1968), when the cultures were grown on various food mixtures. No cyclopiazonic acid was detected.

Ochratoxin A (Table I) and its methyl and ethyl esters are a group of chlorine-containing compounds produced by *Aspergillus ochraceus*, a common fungal contaminant of sorghum and wheat in South Africa which is isolated occasionally from corn. The corresponding chlorine-free compounds are also produced by this fungus, but are nontoxic (Steyn and Holzapfel, 1967). Chemical characterization of ochratoxins A, B, and C (Van Der Merwe *et al.*, 1965), and the synthesis of A and B (Steyn and Holzapfel, 1967) have been accomplished at Pretoria, South Africa. Ochratoxin A has an LD_{50} in ducklings of 500 μg. per kg., a value close to that of aflatoxin. The ochratoxins as well as the aflatoxins are very fluorescent compounds and can be detected quite easily in foods by a thin-layer chromatographic procedure (Eppley, 1967; Scott and Hand, 1967).

Trichoderma viride and *Trichothecium roseum* are common fungi found on corn in Wisconsin. Trichodermin (4-acetoxy-12,13-epoxy-Δ^9-trichothecene) is a metabolite of *T. viride* (Godtfredsen and Vangedal, 1965), but its toxicity to animals has not been reported. Trichothecin (4-isocrotonyloxy-12,13-epoxy-Δ^9-trichothecen-8-one), a metabolite of *T. roseum*, has an LD_{50} of 300 mg. per kg. orally in mice (Freeman, 1955). Other metabolites of *T. roseum* also known to be toxic include rosololactone, rosenonolactone, and the newly characterized 6β-hydroxy-rosenonolactone (Holzapfel and Steyn, 1968).

Fusarium species are also very commonly isolated from moldy corn. *F. equiseti* and *F. scirpi* (*F. roseum*) were examined by Brian *et al.* (1961) and a toxic compound called diacetoxyscirpenol (4,15-diacetoxy-12,13-epoxy-Δ^9-trichothecen-3-ol) was isolated and characterized (Dawkins *et al.*, 1965; Flury *et al.*, 1965). Although originally isolated because of its phytotoxic effects, diacetoxyscirpenol is a potent animal toxin as well (oral LD_{50} is 7.3 mg. per kg. in mice).

In recent studies carried out at the University of Wisconsin, *F. tricinctum* was found to be one of the major toxin-producing species on moldy corn in this area. Several highly toxic metabolites, spiroepoxy derivatives of the trichothecane group, were isolated from *F. tricinctum*. These sesquiterpenoids were formerly called scirpenes, but the trichothecane nomenclature is preferred (Godtfredsen *et al.*, 1967).

Gilgan *et al.* (1966) crystallized a toxin from *F. tricinctum* strain B-24, grown at 7° C., which later (Bamburg *et al.*, 1968a) was found identical with diacetoxyscirpenol. Continuation of these studies with *F. tricinctum* strain T-2, grown at 8° C., resulted in the isolation of a different but closely related substance designated T-2 toxin. This was characterized as 4,15-diacetoxy-8-(3-methylbutyryloxy)-12,13-epoxy-Δ^9-trichothecen-3-ol (Bamburg *et al.*, 1968a). The structures of these compounds are given in Table I, together with some toxicity data. A third metabolite, isolated from strain T-2 grown at 25° C., was 4-desacetoxy T-2 toxin (Bamburg, 1968). All were very active in the rat skin irritation test.

Opening the 12,13-epoxide ring in T-2 toxin and related trichothecanes results in products of greatly diminished toxicity (Bamburg, 1968). However, the number of hydroxyl substituents (at positions 3, 4, 8, and or 15), whether these are free or esterified, and whether or not the Δ^9-double bond is reduced, all have much less influence on the toxicity, although some variations, partly dependent on the method of testing, are observed (Bamburg, 1968).

Application of 0.1 mg. of T-2 toxin in 0.25 ml. of ethyl acetate to the shaved skin on the back of a 21-day-old albino rat produced no noticeable change during the first 12 hours, but after 24 hours the test area was swollen, whiter than the surrounding skin, and sensitive to pressure. Swelling increased the second day and a large scab formed over the treated skin during the third to sixth days. This remained until about the tenth to fourteenth day, after which healing occurred. When animals were given a larger dose (0.25 mg.) of T-2 toxin in the above manner, the white swollen area appeared in 12 to 24 hours, but no further skin symptoms developed. Instead the animals became lethargic, stopped eating, were unable to stand after the second or third day, and most of them died after 60 to 100 hours (Bamburg, 1968). The mechanism of action of the toxin or the exact cause of death have not been determined, but low-dose, long-term feeding experiments with rats and trout indicated that it probably is not carcinogenic (Marasas *et al.*, 1969).

Efforts to elucidate the nature and cause of the crippling fescue foot disease of cattle have led to the suspicion that it may be a mycotoxicosis. A number of common molds found on tall fescue were toxic when tested on rabbit skin (Keyl *et al.*, 1967), and one species, identified as *F. tricinctum* NRRL3249, was extensively studied. Chemical fractionation of the toxic metabolites of this *Fusarium* strain resulted in the isolation of T-2 toxin, plus the butenolide, 4-acetamido-4-hydroxy-2-butenoic acid δ-lactone (Yates *et al.*, 1968). However, it has not been shown that these compounds are actually the cause of the disease.

F. nivali has been studied extensively in Japan recently because it was involved in damage to the 1963 Japanese wheat crop. Cultures grown on rice have yielded three apparently pure toxins, fusarenone, fusarenone X, and nivalenol (Morooka and Tatsuno, 1968; Tsunoda *et al.*, 1968). The chemical structures have not been reported, but the compounds appear to be sesquiterpenoids, possibly of the trichothecane type, although they differ from known members of this group. All produce similar symptoms in mice—viz., marked injury to proliferating cells of the hematopoietic tissue in bone marrow, and to spleen, thymus, lymph nodes, intestinal epithelium, and testis (Saito and Okubo, 1968).

Several lines of evidence suggest that mycotoxins of the trichothecane type may have been responsible to some degree for the widespread human suffering experienced during ATA outbreaks. As previously noted, the fungi thought to be responsible for ATA are now classified as *F. tricinctum*, which produces at least three highly toxic spiroepoxy-Δ^9-trichothecenes (Bamburg, 1968; Bamburg *et al.*, 1968a; Gilgan *et al.*, 1966). The properties of these toxins resemble those reported by Joffe (1962, 1965) and Mayer (1953), in that preparations from the molds implicated in ATA give strongly positive skin irritation tests (rat, rabbit), are stable to long storage at room temperature, are not destroyed by cooking, and are most abundantly produced by mold growth at relatively low temperatures (*ca.* ±10° C.). Furthermore, some of the symptoms elicited by the trichothecane toxins—hemorrhaging in many parts of the body, burning sensations in surfaces contacted by the toxin, e.g., hands, face, gastrointestinal tract (Bamburg, 1968; Mayer, 1953)—are strikingly similar to those reported for ATA. The recently isolated fusarenones and nivalenol from *F. nivali* (Morooka and Tatsuno, 1968; Tsunoda *et al.*, 1968) must also be considered in this connection, as they cause severe damage to the hematopoietic system. Mayer (1953) emphasized that such damage is one of the most important aspects of the pathology of ATA.

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