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Toxic Factors of Mould Origin

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ABSTRACT

The chemistry and effects of mycotoxins associated with human and animal foodstuffs are reviewed. The aflatoxins, metabolites of *Aspergillus flavus*, have been implicated in fatal diseases of farm stock fed on infected peanut cake. Muscarine and the phalloidins are the causative agents in mushroom poisoning. Lysergic acid alkaloids are involved in ergotism. "Yellow rice" toxicity arises from infection with *Penicillium islandicum*, the active principles being islanditoxin and luteoskyrin. Various species of Penicillium, Aspergillus and Fusarium have been linked with other mycotoxicoses and their metabolites characterized. Several fungal metabolites are active hepatotoxins or carcinogens, and the possible etiological significance of mouldy foods is briefly considered, especially in relation to the high incidence of tropical liver disease. Better agricultural practices and more stringent testing to control and detect fungal contamination are advocated.

SOMMAIRE

L'article passe en revue les données chimiques et les effets des mycotoxines existant dans la nourriture de l'homme et des animaux. Les aflatoxines, métabolites de l'*Aspergillus flavus*, ont été en cause dans des maladies mortelles qui avaient frappé du bétail nourri de tourteaux d'arachide infectés. La muscarine et les phalloïdines sont les agents pathogènes de l'intoxication par les champignons. Les alcaloïdes de l'acide lysergique jouent un rôle dans l'ergotisme. Le "riz jaune" devient toxique quand il est infecté par *Penicillium islandicum*, dont les principes actifs sont l'islanditoxine et la lutéoskyrine. On a pu relier d'autres mycotoxicoses à diverses espèces de Penicillium, d'Aspergillus et de Fusarium et identifier leurs métabolites. Plusieurs métabolites fongiques sont des hépatoxines ou des carcinogènes actifs et on examine brièvement la signification étiologique possible d'aliments moisis, étant donné surtout la grande fréquence d'hépatopathies tropicales. On préconise l'amélioration des méthodes agricoles et l'adoption d'épreuves plus strictes pour la découverte et l'élimination de la contamination fongique.

TOXIC factors of mould origin form a branch of study that has recently aroused much interest. Some deleterious activities of moulds have long been recognized, for example, crop losses caused by rusts and blights and the fungal attacks on animal tissues, termed "mycoses". Until recently, little consideration appears to have been given to the possibility that poisonous metabolites produced by the attacking mould might pass into the circulation of the host plant or animal, either affecting organs both distinct and distant from the site of mould growth, or even causing effects at second hand through the host material being eventually eaten as a foodstuff.

"Mycotoxins" is a convenient generic term to describe these substances formed during the growth of moulds, but certain points of difficulty may be mentioned. The word "toxin", although meaning merely a poison, has often tended to be applied narrowly to the bacterial toxins, these forming a class of substances recognized by their complex protein-like nature and antigenic properties. However, no such generalization can be made concerning the chemical nature of mycotoxins, which show a considerable variety of structures, many of them being comparatively simple compounds. Poisoning by mycotoxins is called "mycotoxicosis"; it is frequently mediated through particular organs, notably the liver, kidneys and brain, and is thus to be carefully distinguished from "mycosis", which, as already noted, refers to a generalized invasion of living tissues by actively growing fungi.

Mycotoxins and mycotoxicosis become especially significant in relation to foodstuffs, for these obviously can provide, under suitable conditions, a favourable medium for mould growth, and once the mycotoxins have been formed they remain even though the mould be subsequently killed by sterilization. A good example of this situation is provided by the fungal metabolite, aflatoxin, which has received much study during the last four years and has been largely responsible for the recent upsurge of interest in mycotoxins generally and their recognition as potential hazards. It is therefore proposed to discuss several aspects of the aflatoxin problem in some detail and then to consider more briefly other mycotoxins.

The problem broke in 1960, when approximately 100,000 turkey poults on British farms died from a disease of unknown etiology.[1] At first it was supposed that a virus infection similar to fowl pest

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might be responsible, but increasingly the evidence suggested poisoning. Careful study revealed that all the stricken birds had received feeds containing peanut meal, and that other animals, including pigs and calves, could also be similarly affected by such rations; it was also noted that the suspect feeds all contained meal from a particular shipment imported from Latin America. Possible contaminants such as pesticide residues and alkaloids derived from Senecio or Crotalaria species were soon ruled out by testing; and further work indicated that the toxic factor was associated with the presence of a specific mould, *Aspergillus flavus* (a member of the *flavus-oryzae* group) that had at some stage infected the peanuts from which the meal had been prepared.[2]

Isolation of the fungal metabolites—now named "aflatoxin"—in a state of purity from laboratory cultures soon followed. Four distinct but closely related substances were recognized, referred to as aflatoxin B_1, B_2, G_1 and G_2 respectively, the letters deriving from the colour of the fluorescence (blue and greenish-blue) shown when the compounds were examined chromatographically in ultraviolet light. This fluorescence is intense: it proved invaluable in the preliminary fractionation, and forms the basis of most of the highly sensitive analytical procedures developed for the detection and estimation of aflatoxin.

The chemical constitution of the aflatoxins (Fig. 1) was studied by several research groups, and the structures are now known with certainty.[3, 34-36]

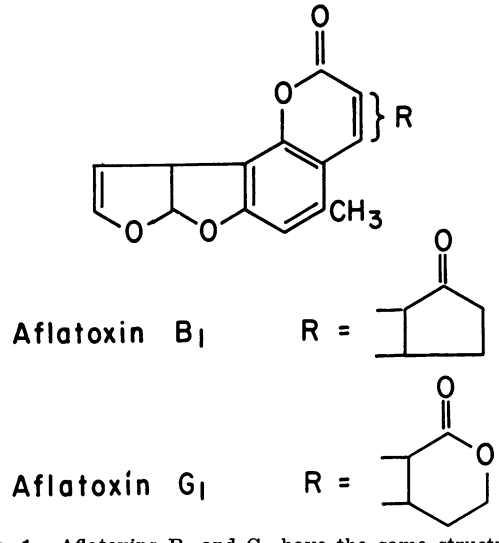

Fig. 1.—Aflatoxins B_2 and G_2 have the same structure as B_1 and G_1, respectively, except that they have no double bond in the outside furan ring.

The four compounds occur in varying proportions according to conditions, the strain of mould and the culture medium being important factors. In natural sources, aflatoxin B_1 seems to be encountered most frequently, the others being either present in negligible amount or absent; but exceptionally B_1 accompanied by significant amounts of either G_1 or B_2 may be found. Under laboratory culture, and especially when related high-yielding mould species are used (e.g. *A. parasiticus*), the proportion of G_1 may rise to over 40% of the complex.

Biologically, the aflatoxins are extremely potent substances and foodstuff contaminations of the order of a few parts per million may be significant, according to the animal species involved. For example, the peanut meal implicated in the turkey catastrophe contained between 7 and 10 p.p.m. (7000-10,000 μg./kg.); but even this apparently small concentration is, in fact, unusually high, and it is probable that for many purposes contamination levels lower by a factor of at least 1000 (i.e. 7-10 μg./kg.) would still be unacceptable. For example, for a day-old duckling of body-weight 50 g., the LD_{50} single dosages of aflatoxin have been given[4] as: B_1 18.2 μg., G_1 39.2 μg., B_2 84.8 μg., and G_2 172.5 μg.

Considering aflatoxin B_1, the most active component, and a peanut meal containing only 1 part per million of it (i.e. 1 μg./g.), it can be seen that less than 20 g. of this meal would contain a median lethal dose. In practical terms, if this meal were incorporated in a ration at a 15% proportion, then 120 g. of this ration, a quantity that might be eaten within a day or two, would likewise contain the LD_{50} dose. Admittedly there are difficulties in attempting to equate the single dose effect with the more gradual intake *via* normal feeding; but from experience it can be said that the quantities suggested would certainly induce considerable distress in the duckling and death would supervene from chronic rather than acute reactions.

In the duckling one characteristic effect of aflatoxin is the abnormal proliferation of liver bile duct cells, a lesion which can be roughly quantified and made the basis of a useful bioassay, while other animals suffering from aflatoxicosis usually exhibit various pathological changes in the liver. Thus it was evident that aflatoxin was an active hepatotoxin. Nevertheless, most of the early observations were necessarily of acute or short-term poisoning cases, and in view of the near-malignant nature of some of the changes observed it was natural to ask what the long-term effects of ingesting sublethal doses of aflatoxin might be.

Answers were soon forthcoming. In 1961 Lancaster, Jenkins and Philip[5] reported that when rats were fed a diet containing 20% toxic peanut meal for six months, nine out of 11 developed multiple liver tumours; it may be estimated that the total intake of aflatoxin was probably between 3 and 5 mg. In other laboratories further trials were carried out with aflatoxin concentrates or pure metabolites, and these all indicated that the substance was an active carcinogen, more powerful, in fact, than benzpyrene or dimethylnitrosoamine. Thus, Barnes and Butler[6] found that about 2.5 mg. fed to rats over a period of three months induced liver tumours, even though after cessation of dosing the rats continued on a normal diet for upwards of a year.

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Such findings showed the aflatoxin problem to be even more serious than had been supposed, because of the now obvious implications for human feeding. Peanuts are grown and consumed in large quantities (over 12 million tons annually) in many parts of the world. Much testing indicated that whilst the peanut meal implicated in turkey deaths referred to previously had contained an unusually high concentration of aflatoxin, its occurrence at some level in peanut products was not exceptional, though the incidence varied widely according to the source and kind of product. Here it may be noted that the production and usage pattern is different in different areas. In the U.S.A. the peanut crop mainly consists of large, high-grade edible nuts which are either eaten whole or made into peanut butter. Not more than a quarter of the crop is pressed for oil and there is no extensive use of peanut meal for animal feeding, as soya, maize and other cereals are plentiful. In tropical countries such as Nigeria, East Africa, India and Burma a smaller type of nut is grown, often under very primitive conditions on small holdings. These nuts are generally pressed for oil (which may be retained in the producing country) and the press-cake is exported, perhaps to Europe, for compounding in animal feeds. Sometimes residual oil in the press-cake may be solvent-extracted, so yielding a peanut meal of low oil content. Britain imports substantial quantities of peanut cake and meal for feeds and uses a good deal of peanut oil as edible fat, but the consumption of edible grade nuts and of peanut butter is comparatively small.

Problems of occurrence, control and prevention may now be considered briefly. In common with all moulds, *A. flavus* requires for growth certain ranges of temperature and humidity. Generally, temperatures above 20° C. (68° F.) and relative humidities exceeding 80% will provide a favourable environment and such conditions are, of course, common in many of the peanut-growing areas. The moisture content of the substrate is also critical, the optimum range for peanut kernels being 15-28%: the mould does not grow on nuts dried to 8% moisture or less.

When first lifted, peanuts contain over 30% moisture, so that slight drying may quickly bring the content within the optimum range, while *A. flavus* is a very common mould whose spores are abundant both in air and soil. Although the intact shell provides a high degree of protection against mould attack, shell damage—whether by termites and insects, plant disease or carelessness during harvesting—is all too frequent. The first defence is thus to dry the nuts as soon as possible after lifting, to a moisture content not exceeding 8%. Mould growth will not then occur, but as spores are still present they will develop, given favourable circumstances. Once dried, therefore, the nuts must be kept dry and not exposed to conditions that might cause their moisture content to rise above the danger level of 8-9%, for example, inadequate protection from rain during transport and storage. Insect attack may also produce local concentrations of water vapour that might assist mould growth. From a more general aspect, attention to other details of agricultural practice may be helpful, e.g. no harvesting of immature or overmature nuts, and care in lifting, handling and decortication, broken nuts being especially susceptible to mould attack.

This outline of a recent and important mycotoxin situation exemplifies several characteristic features: (i) It began as a veterinary problem. (ii) Its true cause was not recognized for some time. (iii) It has human aspects but these are by implication and inference rather than direct.

Touching (i) and (ii) Forgacs and Carll[7] in a comprehensive review devoted mainly to veterinary mycotoxicoses have remarked, "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 be true, the possibility that the source of such toxic chemicals may be fungal in origin is usually ignored. Yet it is well recognized that fungi are among the most potent producers of biologically active organic compounds of great variety and wide spectra." And concerning (iii) the same authors wrote in a later paragraph "The implications of these maladies in human health have been neglected still further." There is much truth in these remarks, but they are probably less true in 1965 than they were three or four years ago when Forgacs and Carll wrote them. It is evident from their review that much good work had been done in the mycotoxin field before 1960, yet there is a strong case for believing that the aflatoxin problem brought into prominence a branch of research that had been somewhat neglected.

From a purely general standpoint Forgacs and Carll[7] and Townsend *et al.*[8] have noted certain useful diagnostic features that characterize outbreaks of mycotoxicosis: (1) The diseases are not transmissible from one animal to another, being neither infectious nor contagious. (2) Treatment with drugs or antibiotics usually has little effect on the course of the disease. (3) In field outbreaks the trouble is often seasonal, as particular climatic sequences may favour toxin production by the mould. (4) Careful study indicates association with a specific foodstuff, e.g. peanut meal, rice or corn. (5) Examination of the suspected foodstuff reveals signs of fungal activity.

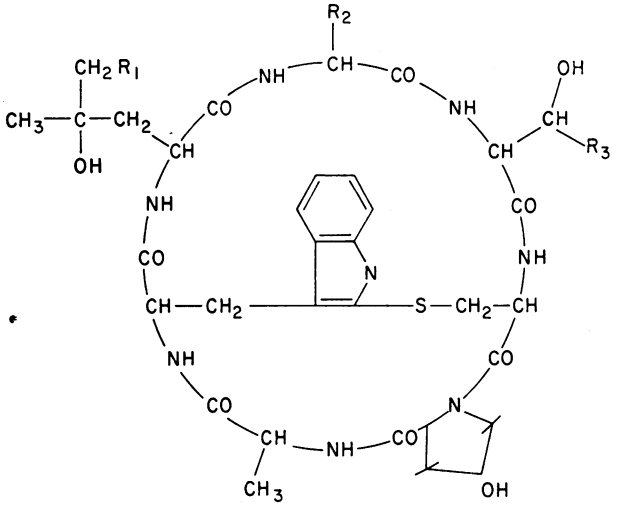
Such criteria are equally applicable to disorders in both animals and man, but it is perhaps worth noting that there are comparatively few examples of *direct* human involvement by mycotoxicosis, that is, cases where the cause of a disorder has been completely established as a mycotoxin.

The following are examples of such direct involvement of humans: (1) poisoning by mushrooms, especially by Amanita spp.; (2) ergotism,

caused by Claviceps infection of cereals; (3) yellow rice toxicity, due to *Penicillium islandicum;* (4) alimentary toxic aleukia (ATA) caused by Fusarium spp.; and (5) dermatitis produced by the pink celery rot fungus, *Sclerotinia sclerotiorum.*

1. *Mushroom Poisoning*

Poisoning by mushrooms is perhaps strictly outside our scope, for this does not develop with the insidious type of effect that occurs when foodstuffs are unwittingly contaminated with small quantities of a powerful mycotoxin: it is essentially a question of misidentification in failing to distinguish between edible and poisonous species. However, the toxins involved are of much interest. The "death-cap" fungus, *Amanita phalloides,* yields three cyclopeptides—phalloidin, phalloin and phallacidin,[9] the structural formulas for which are shown in Fig. 2.



	R_1	R_2	R_3
Phalloidin	OH	CH_2	CH_3
Phalloin	H	CH_3	CH_3
Phallacidin	OH	$CH(CH_3)_2$	COOH

Fig. 2.—Toxins from *Amanita phalloides.*

These compounds are primarily hepatotoxins, but the kidneys, heart and central nervous system are all severely affected. In mice, the LD_{50} intraperitoneal dosage of these compounds ranges from 1.4 to 2.5 mg./kg. Removal of the sulfur bridge or opening of the peptide ring destroys the toxicity. Another poisonous species, *A. muscaria,* contains the quite different muscarine (Fig. 3); although a comparatively simple compound, its structure proved difficult to establish with finality.[10]

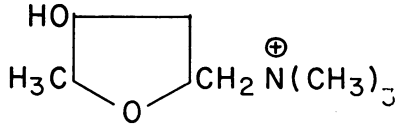


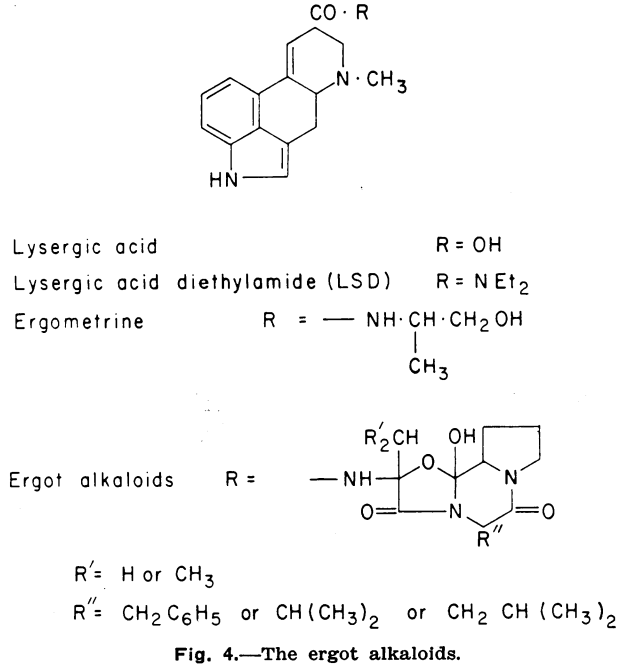
Fig. 3.—Muscarine.

Muscarine is rapid in action and differs from the mycotoxins generally in that it does not bring about severe degeneration in major organs. It behaves like acetylcholine or pilocarpine in stimulating smooth muscle, a reaction that can be suppressed by atropine; thus, in favourable circumstances muscarine poisoning can be successfully treated.

2. *Ergotism*

Ergotism is probably the earliest recorded example of mycotoxicosis; there are some uncertain allusions to it in ancient tablets and sacred books, and more precise descriptions in medieval and later writing. Epidemics occurred in Europe at various times from the 15th to 18th centuries. Characteristic symptoms were gangrene of the limbs and extremities, often accompanied by hallucinations and convulsions and, in pregnant women, abortion. In severe cases the tissues became dry and black, the mummified parts sometimes dropping off. Limbs so blackened like charcoal were believed to have been consumed by the "Holy Fire" or "St. Anthony's Fire", the burning sensations felt by the victims reinforcing this belief. The connection of ergotism with a fungoid disease of rye and other cereals was suspected before 1800, and the life cycle of the fungus was described in the 1850's by Tulasne, who introduced the name *Claviceps purpurea.* Related species such as *C. microcephala, C. nigricans* and *C. paspali* also occur and many cereal grasses, including wheat and oats as well as rye, may be affected.[11, 47]

Ergot is the term applied to the sclerotium of the fungus, and its active principles are alkaloids (Fig. 4) which are all derivatives of lysergic acid, a levorotatory compound; isolysergic acid is the dextro compound.



Lysergic acid R = OH

Lysergic acid diethylamide (LSD) $R = NEt_2$

Ergometrine $R = -NH\cdot CH(CH_3)\cdot CH_2OH$

Ergot alkaloids R =

$R' = H$ or CH_3

$R'' = CH_2C_6H_5$ or $CH(CH_3)_2$ or $CH_2CH(CH_3)_2$

Fig. 4.—The ergot alkaloids.

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Six pairs of alkaloids have been isolated, each pair being based on the two optical isomers of the acid, but only the members of the (—) series are physiologically active. Five of the alkaloid pairs—ergocristine, ergotamine, ergocryptine, ergocornine and ergosine—contain various polypeptide groups attached to the lysergic acid but the remaining one, ergometrine, is much simpler, the substituent being propanolamine. Acute poisoning from ergot is rare, a single dose causing only slight effects, except in the pregnant. On the other hand, the continued ingestion of small quantities, as in bread made from infected grain, eventually produces the classical symptoms of ergotism, as little as 1% in rye being sufficient; inadequate diet intensifies the disorder. Although the cause and control are well understood, outbreaks of ergotism still occur, one having been reported from France in 1953. A recent cross-link is with the hallucinogenic compound lysergic acid diethylamide (LSD), which is being used in the study of certain abnormal mental conditions. Its psychic effects are similar to those produced by mescaline and psilocybine obtained from special types of mushrooms, although chemically the substances are unrelated.

(3) *Yellow Rice*

Toxicity associated with "yellow rice" has been under study in Japan for many years, its connection with mouldiness in the grain being demonstrated as early as 1891[12] during investigations into the cause of beri-beri outbreaks. In later work[13, 37-40] several toxigenic fungi were identified, *viz. Penicillium islandicum, P. rugulosum, P. toxicarium, P. citreoviride* and *P. citrinum.* The first-named organism has been isolated from yellowed rice samples obtained from most of the principal growing areas of the world. Experimentally, it was shown[14, 41, 42] that rice cultures of *P. islandicum* when fed to mice and rats produced severe liver damage, including hepatomas, cirrhosis and proliferation of the bile ducts. The survival times of the experimental animals fell clearly into distinct periods of 100 and 300 days, suggesting a two-stage toxicosis and the presence of more than one mycotoxin. Confirmation was provided by the eventual isolation of two distinct compounds from cultures of *P. islandicum* on synthetic media.[15, 50]

Islanditoxin (Fig. 5), obtained from the metabolism liquor, is an unusual chlorine-containing cyclopeptide shown[16] to be L-seryl-L-seryl-L-dichloroprolyl-D-β-phenyl-β-aminopropionyl-L-amino butyric acid anhydride. It is a powerful hepatotoxin, causing rapid death with severe liver damage and hemorrhage; in mice the LD_{50} dosage ranges between 335 and 655 μg./kg., depending on the route of administration. Removal of the chlorine atoms destroys the toxicity.

The second compound, luteoskyrin (Fig. 5), is quite different, being an anthraquinone derivative; it is 1,1-*bis* (2,4,5,8-tetrahydroxy-7-methyl-2,3-dihydroanthraquinone). As isolated from the mycelium it was accompanied by six related metabolites that were already known from previous work,[17, 51] *viz.* rubroskyrin, islandicin, iridoskyrin, skyrin, erythroskyrin and catenarin: all are highly coloured pigments responsible for the yellowing of the rice. Rubroskyrin is 1,1-*bis* (2,5,8-trihydroxy-7-methyl-1,2-dihydro-4,9,10-anthracene trione). Rugulosin is 1,1-*bis* (2,4,5, trihydroxy-7-methyl-2,3-dihydroanthraquinone) and is a metabolite from the related *P. rugulosum.*[18, 43]

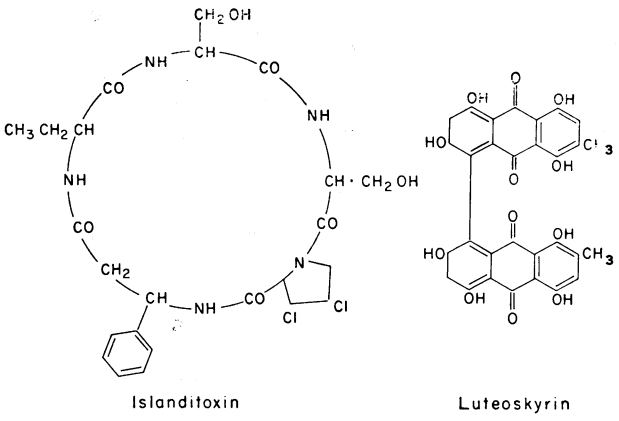


Fig. 5.—Mycotoxins from *P. islandicum.*

Luteoskyrin is likewise a hepatotoxin[15] but is relatively slow acting; death occurs in mice after 2-3 days, the LD_{50} dosages ranging from 147 to 221 mg./kg. There is centrilobular necrosis and fatty degeneration of the liver cells.

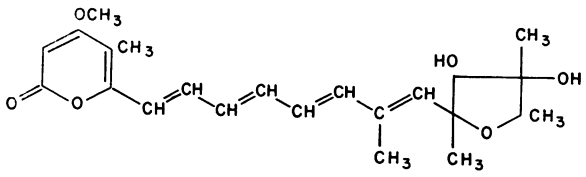
The other moulds isolated from rice also form various toxic metabolites, which may be noted briefly: *P. toxicarium* and *P. citreoviride* yield citreoviridin (Fig. 6), a yellow fluorescent polyene compound.

When given to rats this substance produces paralysis and respiratory failure, and becomes localized in the central nervous system, liver, kidneys and adrenal cortex.[19] The minimum lethal dose in rats has been given as 8-30 mg./kg., depending on dosage route.

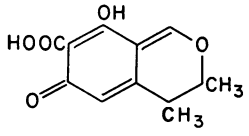
P. citrinum produces citrinin (Fig. 6), a common metabolite that has been isolated from various species of Penicillium as well as from a few aspergilli.

Citrinin produces in mice[20] a nephrosis that interferes with water reabsorption in the kidneys. A related double pyran substance, citreomycetin (Fig. 6), also may be isolated from several penicillia as well as from Citreomyces strains: it is cumulatively rather than acutely toxic, causing chronic kidney damage on prolonged dosing.

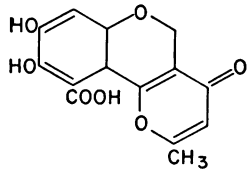
The "yellow rice" problem thus is complex. In many of the principal rice-eating areas there is a high incidence of liver disease and the position is further complicated by the prevalence of vitamin deficiency syndromes such as beri-beri. Because of the delayed onset of many of the symptoms the exact role of mycotoxins is difficult to establish with



Citreoviridin



Citrinin



Citreomycetin

Fig. 6.—Toxic metabolites from rice moulds.

certainty, but the pathological effects observed in animal experiments provide some striking parallels with those found in human disorders.

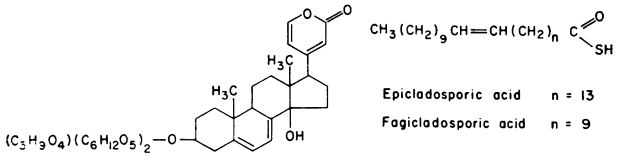
4. *Alimentary toxic aleukia (ATA)*

This serious toxicosis develops after eating overwintered mouldy grain. It is characterized by profound changes in the blood, including leukopenia, agranulocytosis and exhaustion of the bone marrow. Although often fatal, recovery is possible under skilled treatment and nursing.

ATA has broken out in Russia at various times since it was first reported from Eastern Siberia in 1913. Severe epidemics occurred between 1941 and 1947, the incidence of toxicity exceeding 10% of the population in the most seriously affected areas with many fatalities. Considerable study has been given to the problem since 1932, and over 240 papers have been published (in Russian) on the subject, but many of them are not readily accessible. The comprehensive review made in 1953 by Mayer[21] is therefore of great value, while shorter and more recent accounts have been given by Forgacs and Carll[7] and Joffe[22, 48]

It seems to be now accepted that the moulds chiefly responsible are fusaria, especially *F. sporotrichioides* and possibly *F. poae* and *F. lateratium.* However, there are suggestions that Cladosporium and Alternaria species among others may also be involved. The fungi infect cereals, millet in particular, that have been allowed to overwinter on the ground. Mild winters with much snow, succeeded in the spring by frequent alternations of freezing and thawing, appear to be favourable as regards mould growth: severe winters followed by a rapid thaw and drying weather are unfavourable. The fungi are very resistant to cold and still grow slowly even at —10° C.; conditions for toxin formation occur during the early spring when temperatures are between 1° and 4° C. and the moulds are at the sporulating stage. About 3 lb. of infected millet eaten over six weeks may be enough to induce pathological changes in the hematological system. Toxicity is not destroyed by cooking but milling processes may remove some of the infected portions and make the grain harmless.

Work has been done on the production and isolation of the toxins involved, and certain of the symptoms have been reproduced by culture extracts in experimental animals. Three active principles have been described: fusariogenin glycoside, epicladosporic and fagicladosporic acids (Fig. 7).

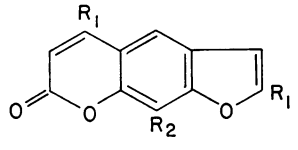


Fusariogenin glycoside — The Cladosporic acids

Fig. 7.—Fungal metabolites associated with ATA.

5. *Pink rot dermatitis*

The fungus *Sclerotinia sclerotiorum* causes "pink rot" on celery stems. When this growth comes into contact with the hands during gathering it sensitizes the skin, and on exposure to light, dermatitic lesions develop.[23] The active principles are 8-methoxy- and 4·5·8-trimethylpsoralen (Fig. 8).



	R_1	R_2
8 – methoxypsoralen	H	OCH_3
4·5′·8 – trimethylpsoralen	CH_3	CH_3

Fig. 8.—The psoralens.

The compounds have been known for some time, having been isolated from various higher plants, e.g. *Psoralea corylifolia* and *Ammi majus.*

The foregoing paragraphs have mentioned some of the principal mycotoxins affecting human foods. As indicated earlier, much of the work on mycotoxicosis has been in the veterinary field, and there have been various descriptions of animal disorders associated with the consumption of mouldy straw, hay and cereals. A few examples of these will be given to illustrate the range of organisms and variety of metabolites encountered.

A peculiar disease affecting horses has been associated with the fungus *Stachybotrys atra* which infects their fodder. The toxicosis occurs in two forms, typical and atypical, and there are various pathological changes in the blood, hemorrhages and tissue necrosis. Recovery from the typical form is not uncommon but the rapid, atypical form has

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a high mortality. Other farm animals are equally susceptible, while the infected straw can induce dermatitis in the farm workers.[7] A so-called "estrogenic syndrome" in swine, marked by abnormalities in the reproductive and mammary organs, has been associated with the fungus *Monascus papulospora*, which grows on corn and apparently forms estrogenically active metabolites.[24]

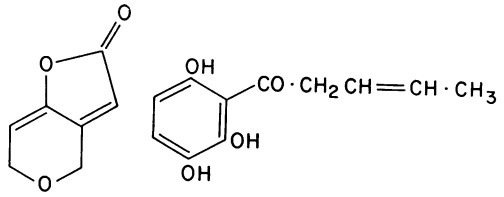

Fig. 9.—Patulin and maltoryzine.

A loss of over 100 dairy cattle in Japan in 1952 was attributed[25] to the consumption of mouldy feeds bearing strains of *Penicillium urticae*, which produce patulin, a pyrone derivative (Fig. 9). This is a common metabolite of *P. patulum* and other species of both penicillia and aspergilli, and is a neurotoxic substance. A further outbreak of toxicosis in Japanese cattle two years later was traced to malt feed containing *Aspergillus oryzae* var. *microsporus*. This yielded a new metabolite, maltoryzine, elucidated as 1-(3-pentenoyl)-2,3,6,-trihydroxybenzene (Fig. 9).

On injection into mice it caused tremors, anorexia and acute muscular paralysis; hemorrhages of the brain and serous membranes were found post mortem.[26, 44] The closely related *A. flavus* has also been reported to yield a similar tremorigenic substance.[27]

Considerable attention has been given in several countries to mouldy corn toxicoses, involving a range of animals and various disorders.[7] Two implicated organisms in particular, *Penicillium rubrum* and *Aspergillus chevalieri*, have been the object of study. From cultures of *P. rubrum*, a hepatotoxic substance[28, 49] was obtained. Townsend *et al.*[8] have recently made further progress in the isolation and characterization of this substance; it appears to contain hydroxyl, γ-lactone and carboxyl groups. From a toxigenic strain of *A. chevalieri* Townsend *et al.*[8] obtained a toxic principle which, by spectral data, was shown to be identical with the unusual isocyanide, xanthocillin (Fig. 10), already known as a metabolite of *P. notatum*.[29, 45]

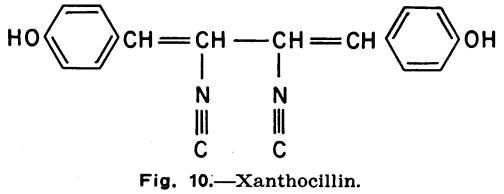

Fig. 10.—Xanthocillin.

This substance has antibiotic properties but is too hepatotoxic for use.

New fungal metabolites called ochratoxins have very recently been isolated from maize cultures of *Aspergillus ochraceus*. This common mould sometimes invades stored wheat and also enters into fermentation processes for fish ("katsuobushi").[30] Ochratoxin A (Fig. 11) is an amide formed from

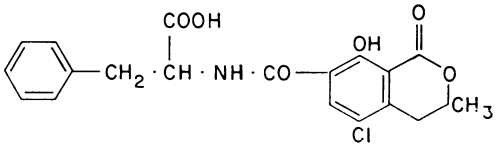

Fig. 11.—Ochratoxin.

phenylalanine and a substituted chloroisocoumarin. It is a highly toxic substance comparable in potency with the aflatoxins, the LD_{50} for ducklings being 500 μg./kg. (aflatoxin B_1 560 μg./kg.). The liver cells of the birds showed gross fatty infiltration but not the necrosis or bile duct proliferation characteristic of aflatoxicosis. Ochratoxins B and C are respectively the de-chloro and ethyl ester derivatives of the A compound; both are non-toxic.

It needs to be stressed that the various examples of animal toxicoses should not be considered in isolation or dismissed as of importance only to veterinarians. All such problems may have a bearing on human nutrition or disease. In the lowest terms, fatal poisoning of farm animals by mycotoxins represents a loss of potential food; but even their survival may carry an insidious menace. Cows fed peanut meal containing aflatoxin excrete in their milk a toxic factor which produces in ducklings characteristic liver lesions indistinguishable from those caused by aflatoxin itself.[31, 46] Certain mycotoxins may concentrate in animal organs, such as liver and kidneys, that are eventually utilized as food.

When the wide spectrum of moulds and the variety of their metabolites are considered, there at once arises the possibility that these agencies may have etiological significance for many diseases of doubtful origin. For example, Oettle[32] has reviewed the epidemiology of liver carcinoma, which in certain areas of Africa and the Far East has an exceptionally high incidence. Although readily conceding that the evidence was still far from adequate, he concluded that mycotoxins offered a plausible explanation, correlating the observed distribution better than did malnutrition, bilharzia, infective hepatitis and chronic alcoholism: in particular, he noted that the moulds require for growth high relative humidities (over 80%) and that most of the regions concerned provided these conditions as well as harbouring primitive food storage methods that would favour mould attack.

It is surely evident that a new and serious approach to the whole question of fungal contamina-

tion of foods is required. There have been tendencies in the past to regard moulds in food as unfortunate nuisances, unesthetic certainly, but basically harmless; commodities deemed unfit for human consumption might nevertheless still be used as cattle-feed. Fortunately, some far-sighted authorities have for long held that all mould and filth is potentially harmful and have framed appropriate standards. In bringing foods up to these high standards there will have to be much attention to details of agricultural and storage practice, as well as suitable inspection schemes. One problem will be that of devising test methods. With aflatoxin we have been fortunate: it is one of the most potent mycotoxins known, but nature has, in compensation perhaps, also endowed it with those intensely fluorescent qualities that make detection and estimation at extremely low levels perfectly feasible. Current analytical methods, such as those developed by the Tropical Products Institute,[33] will pick up amounts as small as 0.0004 μg. (= 0.4 ng.). The toxicological significance of the recently discovered ochratoxin is not yet clear, but this compound is also highly fluorescent and analytical methods of comparable sensitivity will no doubt be evolved if required.

It may be that methods will be needed only for two or three important mycotoxins. Aflatoxin would certainly be one of these, for although it may be primarily thought of as a peanut problem, the causative organism, *Aspergillus flavus*, is widespread and many strains of it will form aflatoxin when grown on a variety of substrates — for example, maize, cottonseed meal, palm kernels and brazil nuts. On the other hand, it is equally possible that further research will continue to uncover new toxic metabolites of real significance. There can be no guarantee that all these will be equally obliging in possessing fluorescent or other properties for their easy detection — quite apart from the inconvenience of having to apply a whole battery of chemical procedures to one commodity. Attention therefore may increasingly turn to the basic problem, that of detecting or assessing the mould growth itself in foods and feeding-stuffs. New sampling and examination techniques may be necessary, with confirmation in selected instances by chemical or biological assay.

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