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Mycotoxins: Toxicity, Carcinogenicity, and the Influence of Various Nutritional Conditions*

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Toxicologic diseases of man and animals, associated with molds growing on foods, have been recognized for centuries. Only in recent years, however, have these mycotoxicoses received the attention of many laboratories and skilled scientists around the world in a broad interdisciplinary effort.

This review covers the literature on mycotoxicoses but centers on those about which most is known, particularly the diseases associated with metabolites elaborated by some strains of *Aspergilli, Penicillia, Fusaria, Stachybotrys, and Claviceps.*

The ubiquitous nature of the aflatoxins, toxic metabolites produced by *Aspergillus flavus,* make them important to public health, especially since it is now known that certain areas of endemic liver disease coincide with consumption of aflatoxins and, often, malnutrition.

The older disease of ergotism, the scourge of Europe for centuries, is considered in detail. Alimentary toxic aleukia, which has caused enormous suffering in Russian human and animal populations, is better understood as a result of relatively recent experimental investigations. Stachybotryotoxicosis, a disease previously considered to be of significance only to man has now been identified in domestic animals

Finally, Japanese studies have clearly revealed the hepatotoxicity of certain metabolites of *Penicillium* molds.

Factors that influence susceptibility to mycotoxins and the hazards they present to man are also reviewed.

Introduction

Metabolites of certain molds growing on foodstuff have caused toxic diseases in man and animals throughout recorded history; but an understanding of the relationship between the molds, the food, and the diseases has begun to develop only recently.

Since the turn of the century, it has been known that certain fungi produce toxic metabolites eliciting biologic responses in both man and animals. As early as the 19th century, a disease associated with the consumption of yellow, discolored rice was recognized in Japan and was established as a toxicologic entity. Similarly, alimentary toxic aleukia (ATA), associated with overwintered wheat, affected both man and animals in Russia and was known for many decades. Centuries prior to these observations, ergot poisoning through ingestion of flour and bread contaminated with a fungus created widespread epidemics of ergotism in Europe—known to the ancients as St. Anthony's fire. Thus, ergot poisoning was recognized retrospectively as the first of many mycotoxicoses.

Despite the early recognition of ergot poisoning and the toxicoses associated with yellow rice and overwintered grain, the mycotoxicoses

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remained generally neglected diseases until about 1960. A serious outbreak of a toxic turkey disease in England precipitated scientific investigations which delineated the nature of the disease presently known as aflatoxicosis. This incident led to the realization that mycotoxins, and especially aflatoxins, presented a serious threat to public health as well as to animal economy; large numbers of investigators and vast resources were drawn into investigation of the problem; and resultantly, during the past decade an enormous amount of literature in the field has accumulated. This large volume of literature is somewhat misleading by subtly suggesting a long-standing knowledge; actually the field of mycotoxin research is barely out of its infancy.

Surveys of foods and feeds around the world have revealed that the problem of mycotoxicosis is not limited to any one geographic area but is a real or potential problem in all areas where molds grow *(1)*. In fact, virtually all staple food products consumed throughout the world are subject to contamination by mold toxins. Observations that some of the mycotoxins are carcinogenic in certain animal species and furthermore that they are associated with a high incidence of liver cancer in some human populations have added considerable impetus to research efforts.

The huge amount of literature on mycotoxicoses cannot be covered in a relatively short review; therefore, this treatise centers on the salient features of the diseases associated with a few of the most commonly encountered molds—*Aspergilli*, *Penicillia*, *Fusaria*, *Stachobotrys*, and *Claviceps*. Exclusion of other molds and even of some of the toxins produced by those listed above does not imply that they are not important, but rather that limitation of both time and space prevent their inclusion. In addition to pointing out the major toxic fractions that are known to be produced by the molds, other relevant information concerning the mold metabolites will be presented. Furthermore, factors that may influence biologic response to the toxins will be discussed, particularly emphasizing nutrition. With the exception of aflatoxin research, investigation of nutritional influences consists to a large extent only of clinical observations.

Ergot

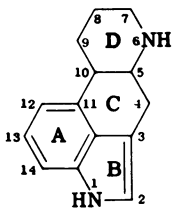
During the Middle Ages, large-scale epidemics of a toxic disease occurred in middle and western Europe *(2)*. Although the causative agent was not known, it was recognized that the disease was associated with rye used for making bread. Two characteristic forms were described: gangrenous and convulsive ergotism. There are some hints in the older literature that ergot may have been used as a medicinal; its actual use as a drug was described for the first time in 1582 *(3)*.

Ergot is composed of several true alkaloids, some of which have been thoroughly investigated. The best-known genus of fungi capable of forming ergot alkaloids is *Claviceps purpurea*, one of the first species known to produce mycotoxins; it is most often found as a parasitic fungus on rye and several wild grasses, causing the disease ergotism *(4)*.

In about 1875, the first crystalline alkaloid preparation was isolated from ergot by a French pharmacist, and in 1918, Stoll described the first homogeneous, chemically pure alkaloid which exhibited the typical biologic properties of ergot and which was designated as ergotamine *(3)*.

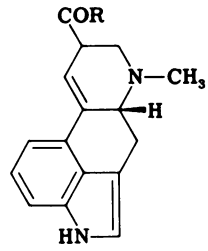
The highly variable composition of the ergot alkaloid mixture and the complex chemical structure of the bases impeded the further evaluation and elucidation of ergot alkaloids *(5)*. The ergot alkaloids are 3,4-substituted indole derivatives, for example, ergoline (I), lysergic acid derivatives (II), and clavine alkaloids (III). Many ergot alkaloids can be derived from lysergic acid, but the main alkaloids of *C. purpurea* are peptides. On hydrolysis, these peptides (IV) decompose into lysergic or isolysergic acid, ammonia, a keto acid, and two amino acids. Proline is the amino acid common to all peptide alkaloids; the various alkaloids differ from each other mainly in the type of the second amino acid obtained upon hydrolytic cleavage. There are many additional chemical compounds derived from alkaloids which cannot be covered in this review; for further information, the reader is referred to Gröger *(3)*.

During recent years, the biosynthesis of ergot alkaloids has been under intensive investigation. The ergoline ring system in ergot fungi as well as in other plants is constructed from tryptophan and mevalonic acid. The *N*-methyl group



Ergoline

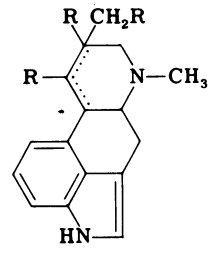
I



Lysergic acid derivatives

R = Tripeptide or smaller units

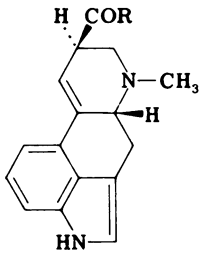
II



Clavine alkaloids

R = H = OH

III



IV

R = NH_2: Ergine

R = $NH \cdot C(H)(CH_3)(OH)$: Lysergic acid methylcarbinolamide

R = $NHC(H)(CH_3)(CH_2OH)$: Ergometrine (ergobasine)

R = $NHC(H)(CH(CH_3)_2)(COOCH_3)$: Lysergic acid-L-valinemethylester

is derived from methionine by a transmethylation reaction. L-Tryptophan is the immediate precursor of the ergoline nucleus. During the incorporation of tryptophan into ergoline at the alpha carbon atom of the side chain, an inversion of configuration occurs; an isoprene unit also may participate in the formation of the alkaloid—ergoline. The complicated chemistry of the alkaloids has been described in great detail by Gröger *(3, 5)*.

The particular interest in chemical assays for ergot alkaloids and their derivatives arises from their therapeutic and toxic properties. Many methods have been described for the qualitative and quantitative assessment of the compounds. Lysergic acid derivatives are unstable, usually decomposing upon exposure to light or oxidizing agents; the decomposition to isolysergic acid derivatives destroys the pharmacological effect, complicating assays of the active principles in crude drugs. Characteristic color reactions occur when the ergot alkaloids are exposed to sulfuric acid or *p*-dimethylaminobenzaldehyde in sulfuric acid. Through the addition of ferric chloride, this color reaction—known as the van Urk reaction—has been improved and standardized for the quantitative measurements of ergot alkaloids *(6)*. Derivatives of lysergic acid, isolysergic acid, and some other compounds produce an intense blue color in these tests. Ergolines are also quantitatively assessed by titration, spectrophotofluorometric methods, and by ultraviolet absorbance *(7)*. Spectral data about infrared radiation and neutron magnetic resonance (NMR) are known for many of the alkaloids. Thin-layer chromatography as well as paper chromatographic methods have been used for further separation of the various alkaloids *(8, 9)*.

The ergot alkaloids have been used as therapeutic agents for many years *(8)*. According to the sites of action—periphery, neurohumoral system, central nervous system—the six principal effects of all natural ergot alkaloids can be categorized as follows. Vasoconstriction and contraction of the uterus are perhaps the most important peripheral effects; the classic use of ergot alkaloids in obstetrics arises from this influence. Principal neurohumoral effects appear as antagonisms to serotonin and adrenaline. Effects of ergot on the central nervous system are many and varied, including a depression of the vasomotor center and a stimulation of sympathetic structures in

the midbrain, particularly in the hypothalamus.

Slight modifications in the chemical structure of an alkaloid can produce pronounced changes in biological activity. Thus, certain compounds are used therapeutically in peripheral and cerebral vascular disorders, while others are used in essential hypertension.

The interest and the amount of research associated with lysergic acid diethylamide (LSD) is reflected by more than 2000 scientific and popular publications during the last several years *(2, 10)*. As the prototype of hallucinogens, LSD has been an object of recent fascination *(10)*.

The ergot alkaloids reportedly influence pregnancy *(11)*, perhaps by affecting progesterone metabolism; because of its potential application to contraception, this area is currently under intensive investigation.

Fusaria and Alimentary Toxic Aleukia

Probably since the 19th century, outbreaks of an enigmatic toxic disease occurred in Eastern Siberia and the Amur region. As a result, the Soviet government established the Institute of Epidemiology and Microbiology of the U.S.S.R. within the Ministry of Health, including a special laboratory for investigating all aspects of the disease *(12, 13)*. Starting during the spring of 1932, when the same disease reappeared suddenly in endemic form throughout several districts of Western Siberia, the laboratory conducted comprehensive studies of the disease, its etiologic agent(s), and its control.

The signs and symptoms of the disease, called alimentary toxic aleukia (ATA), have been described repeatedly in the Russian literature. The signs include fever and hemorrhagic rash as well as bleeding from gums, nose, and throat; signs and symptoms also comprise necrotic angina, extreme leukopenia, agranulocytosis, and an exhaustion of the bone marrow with general sepsis. Mortality has always been high. Mostly occurring in agricultural areas, the disease severely affected whole families and even entire villages.

Although the disease initially received many names, including septic angina, alimentary mycotoxicosis, alimentary hemorrhagic aleukia, aplastic anemia, hemorrhagic aleukia, and agranulocytosis, a committee of the Soviet Health Ministry concluded that the most appropriate term was alimentary toxic aleukia (ATA).

At first the disease was considered to be infectious, but neither bacteriological studies nor epidemiologic investigations confirmed the hypothesis. Alternatively, the disease was considered a vitamin deficiency or a poisoning through bacterially contaminated food; neither of these hypotheses was substantiated. The various false concepts delayed recognition of the true nature of ATA. Eventually it was realized that the disease stemmed from an ingestion of overwintered grains infested by toxic fungi. These grains formed the staple diet of the peasant population in agricultural areas of Russia.

ATA occurred with special severity during World War II, reaching a peak in 1944, when the population of the Orenburg district alone suffered an alarming number of casualties *(12)*. The morbidity among the population in this district exceeded 10% and a high mortality occurred in 9 of the 50 counties. Occurrence of the disease was related to the particular situation prevailing in some parts of the Soviet Union at that time; because of famine, the population was driven to collect grains that had been left in the field under the thick snow cover of winter. Under ordinary circumstances the wheat would have been gathered earlier; but the shortage of manpower occasioned by the war prevented harvesting at the proper time.

The role of toxic fungi as an etiologic agent in ATA was examined extensively in the Institute of Epidemiology and Microbiology in Moscow. From more than 1000 samples of overwintered grains, the institute isolated over 3500 fungal cultures belonging to 42 genera and 200 species. Among the isolates, 61 showing *Fusarium poae* and 57 showing *Fusarium sporotrichioides* were toxic to animals. Numerous investigations in different laboratories supported these results; between 1943 and 1949, trial plot experiments determined the most favorable conditions for toxin production in overwintered wheat.

The extensive work conducted in the Soviet Union established that the disease is caused by toxic metabolites stemming from those species of *Fusaria* which grow on grains that are harvested after the snow has melted in the spring (Table 1); evidence suggests that the toxin is produced during the spring thaws. The follow-

Table 1. Toxicity of *Fusarium* fungi isolated from overwintered cereals, summer-harvested cereals, and their soils.[a]

Fungus	Number of isolates											
	Overwintered cereals: grains and vegetative parts				Soil				Summer-harvested cereals: grains and vegetative parts			
	Toxic	Mildly toxic	Non-toxic	Total	Toxic	Mildly toxic	Non-toxic	Total	Toxic	Mildly toxic	Non-toxic	Total
Fusarium arthrosporioides Sherb.	—	1	7	8	—	—	2	2	—	—	5	5
F. avenaceum (Fr.) Sacc.	3	3	26	32	—	—	10	10	—	—	3	3
F. culmorum (W. G. Sm.) Sacc.	2	1	13	16	—	—	—	—	—	—	—	—
F. equiseti (Cda.) Sacc.	7	3	41	51	—	1	9	10	—	—	27	27
F. graminearum Schw.	—	1	2	3	—	—	—	—	—	—	—	—
F. javanicum Koord.	—	—	8	8	—	—	—	—	—	—	5	5
F. kühni (Fuck.) Sacc.	—	1	9	10	—	—	—	—	—	—	—	—
F. lateritum Nees	2	2	24	28	—	1	3	4	—	—	—	—
F. moniliforme Sheld.	1	3	22	26	—	1	10	11	—	—	—	—
F. nivale (Fr.) Ces.	—	2	11	13	—	—	—	—	—	—	—	—
F. oxysporum Schl.	1	2	16	19	—	1	13	14	—	—	2	2
F. poae (Pk.) Wr.	44	17	2	63	2	3	—	5	—	—	—	—
F. redolens Wr. Wr.	1	—	5	6	—	—	2	2	—	—	—	—
F. sambucinum Fuck.	1	1	14	16	—	—	—	—	—	—	—	—
F. semitectum Berk. et Rav.	2	2	23	27	—	—	—	—	—	—	—	—
F. solani (Mart.) app. et Wr.	—	3	16	19	—	—	5	5	—	—	—	—
F. sporotrichioides Sherb.	42	15	4	61	2	2	—	4	—	—	2	2
F. tricinctum (Cda.) Sacc.	2	1	19	22	—	—	5	5	—	—	—	—

[a] From Joffe *(13)*.

ing specific case serves as a typical example. When harvested in autumn and winter before the snow melted, grains were either nontoxic or only slightly toxic; when harvested after a relatively mild winter with abundant snow followed by frequent spring freezing and thawing, often grains were highly toxic. The disease usually appeared after victims had eaten only 2 kg of overwintered grain, and death resulted from the eating of as little as 6 kg. Signs and symptoms generally appeared within 2 to 3 weeks after ingestion; death occurred 6 to 8 weeks after the uptake of large toxin amounts.

Populations receiving balanced diets were much less sensitive to the toxins than populations subsisting mainly on overwintered cereals *(13)*. Additional factors influencing the response to the toxins included the kind of cereal which was ingested, the altitude, and the thorough washing of grains in boiling water before grinding. Prosomillet and wheat were the cereals most likely to be toxic (Table 2); high altitude appeared to decrease the incidence of the disease in several population groups; and the degree of toxicity was considerably diminished through thorough washing of the grains in boiling water which, as shown by later studies, removed some of the toxins.

Biological and chemical properties of the fungi responsible for ATA are known. The evidence points conclusively to the *Fusarium* molds as primary agents, but climatic conditions also enter the toxicological problem. An excellent and comprehensive discourse on ATA is presented by Joffe *(12, 13)*.

Data on the relative toxicities of barley, millet, and wheat during several years following the serious outbreaks in the early 1940s are compiled in Table 2. Although wheat was most toxic, millet caused a higher incidence of disease; millet was consumed by a much larger number of people, and it was widely grown in areas where other conditions contributed to a high incidence. Furthermore, the millet ripened too late to be harvested before winter and early spring.

Several other conditions were studied in detail: persistence of toxicity in stored grain, role of the soil in the process of toxin formation, toxicity of both vegetable cereal parts and soil, meteorological conditions, seasonal effects, as well as a comparison between toxicity of fungal cultures and cereal samples.

A particularly interesting result of the research on *Fusaria* is the immunization associated with toxic cultures. If investigations confirm the hypothesis that an immunity can be conferred on individuals, an exciting new area will be opened to research in the field of mycotoxicoses.

Evidence from extensive investigations clearly demonstrates that the strains of *Fusaria* vary greatly in their capacity to produce toxic materials *(14, 15)*. Among the many factors influencing toxicity are ecological conditions, particularly the substrate on which the fungus grows. The best nutrient sources for fungal growth are carbohydrates, including starch and glucose, along with peptone and asparagine as suppliers of nitrogen. Ammonium sulfate and

Table 2. Toxicity of samples of various cereal crops, 1944–1949, from the Orenburg District.[a]

Degree of toxicity	Millet		Wheat		Barley	
	Number of samples	%	Number of samples	%	Number of samples	%
Toxic	11	2.6	19	4.6	5	1.9
Slightly toxic	22	5.2	19	4.6	14	5.5
Nontoxic	387	92.2	377	90.8	236	92.6
Total number of samples examined	420		415		255	

[a] From Joffe *(13)*.

sodium nitrate also enhance the production of toxins. The acidity of the medium, too, is important; the most suitable pH values lie between 4.6 and 5.4.

The toxins of *Fusarium* cultures produce both localized and general effects *(12)*. Localized effects, as evidenced by skin tests, are restricted to necrosis of the skin; these confined effects provide a convenient biologic assay. General effects comprise defective blood production, hyperemia of the digestive tract, and acute organ degeneration. Under practical conditions, skin testing is now used as a reliable technique for toxicity determination of overwintered grains.

Guinea pigs and rabbits are most useful for bioassays. In addition to skin responses, these animals also exhibit a severe depression of leukocytic bone marrow elements together with a general depression of the hemopoietic system.

The clinical picture of ATA parallels strikingly the signs and symptoms as well as the bone marrow depression observed in stachybotrys toxicity. The first, second, and third stages of ATA in human patients closely resemble the first, second, and third stages of stachybotryotoxicosis in animals. Local manifestations are burning sensations in mouth, esophagus, and stomach, General manifestations are vomiting and depression of leukopoiesis, erythropoiesis, and thrombopoiesis. Although ATA depresses the hemopoietic system, the bone marrow tends to remain viable. Based on these observations, several investigators assume that the toxins associated with overwintered cereals do not act primarily on the bone marrow but on other tissues which regulate the hematopoietic system, the autonomic nervous system, and the endocrine system.

In ATA, several investigators observed changes of the nervous system such as impaired reflexes, general hyperesthesia, cerebral hemorrhages, encephalitis, and destructive lesions in the sympathetic ganglia.

The only prophylactic measure against ATA consists in the elimination of toxic grain from food.

Stachybotryotoxicosis

A disease of then unknown etiology, but apparently of a toxic nature, affected horses throughout the Ukraine in 1931 *(16)*. The disease was characterized by an unusually high mortality rate and had clinical and pathological histories which were unlike any disease entities previously reported. The early studies of Drobotko and colleagues *(17)* established that the disease was neither contagious nor infectious; unaffected horses were stabled adjacent to sick ones without contracting the disease, and attempts to transmit the disease by other methods failed also. Review of many cases revealed a relationship between straw used as roughage or bedding and the appearance of the toxic disease. It required several years and the combined efforts from several medical disciplines to establish the causative agents as metabolites of the fungus *Stachybotrys alternans*. Working together, veterinarians and microbiologists established the etiology, described the clinical symptoms in detail, and recommended therapeutic measures as well as procedures for preventing the disease. Conclusive evidence of the toxicity of the material produced by the fungus was established by culturing the mold on artificial media and feeding it to horses. After being fed sufficient amounts of this material, the horses developed signs, symptoms, and lesions which were identical to those observed in the field. Typically there was an inflammatory response in the oral mucosa along with edema of the lips; leukopenia was always observed.

Following these studies, the disease appeared in several other areas of Russia and in parts of eastern Europe as well. Over a 10-year period, the toxicoses attained enzootic proportions and then seemed to subside, with only sporadic cases appearing in those areas where it had previously been enzootic. Reports of the disease were not available during World War II, but since the War, occasional outbreaks have been reported in several regions *(18)*.

The Russian literature included statements that the horse was the only large animal susceptible to *Stachybotrys* toxin; however, in the United States, Forgacs and co-workers observed the toxicosis in swine, sheep, and calves as well as in horses *(19)*. A natural outbreak of stachybotryotoxicosis in cattle has been reported; it was associated with straw contaminated by the fungus *(20)*. More recently the disease has been produced experimentally in

laboratory animals including dogs, rabbits, guinea pigs, and mice *(21)*; chicks, too, were sensitive to *Stachybotrys* toxin. In Russian experiments, human volunteers were exposed to aerosols containing toxic strains of *S. alternans* or substrate infected with these strains; the patients developed systemic and localized toxic manifestations *(20)*.

Studies on the epizootiology of the disease indicate that the first cases in horses usually appear in the fall when the animals are stabled and when fodder, hay, or straw make up a considerable portion of the diet. The number of affected animals increases as winter passes on, usually reaching a peak about February and March. The disease generally subsides when the horses are turned out to pasture. As with other mycotoxicoses, there is no immunity conferred upon the host; when the disease occurs on a particular farm, it is likely to recur, even in the smaller animals. When the disease appears on a farm, the number of affected horses quickly rises within one week to 50% or more of the entire stock; during the second week usually all of the animals are sick. There is also a peracute form of the disease in which entire herds can be violently affected within 6 or 8 hr; such cases are exceptional, however. The toxin does not appear to be transmitted through the milk of the mother, since colts nursing affected mares do not develop the disease. Age, breed, sex, physical condition, work load, and other factors apparently have no influence on the severity or course of the disease. Although mortality may be moderate in certain cases, it usually is high; the highest rate appears in the peracute forms, in the chronic form, and in animals suffering from various degrees of malnutrition.

The typical form of the disease *(18)* occurs in animals with a continuous exposure to low levels of the toxin. The initial manifestation appears as stomatitis; necrosis is obvious about the mouth, particularly in the wrinkles of the skin at the mucocutaneous junction. The stomatitis then progresses to bleeding, swelling of both upper and lower lips, and excessive salivation along with enlargement of the submaxillary lymph nodes. Temperatures may increase only 1–2°C, and the blood may show a transitory neutrophilia. The duration of this early stage generally varies from 1 to 3 weeks; despite continued ingestion of sublethal amounts of the toxin, the local lesions then gradually subside and the animals enter an apparent period of clinical remission. During this period, however, thrombocytes decrease markedly, clot retraction time increases, and the blood ultimately fails to coagulate. During the same period, leukopenia and agranulocytosis develop, the leukocyte count dropping to 2000/cm^3 of whole blood or less. Intestinal disturbances of various degrees, including atony, generally appear during this the second phase of the disease. The third stage is characterized by rising body temperatures, leukopenia, and severe thrombocytopenia. The leukocyte count often decreases to 100 white cells/cm^3 of whole blood. The animal develops a weak pulse and often arrythmic heartbeat, along with disturbances of the alimentary tract. Pathologic changes are characteristic: necrotic areas appear on the mucous membranes of cheek, gum, tongue, soft palate, and lips; the blood glucose level drops to about 50% of normal; the serum bilirubin level increases; and the inorganic phosphorus content declines by a factor of 10 or more. This final stage lasts from 1 to 6 days and usually terminates in death.

An atypical form, having many of the signs, symptoms, and lesions characterizing the typical form, also occurs. The main syndrome comprises nervous disorders; loss of reflexes, hyperirritability, hyperesthesia, loss of vision, and inability to move about. Despite anorexia, the horse continues to drink large quantities of water even though it swallows with considerable difficulty. Death often ensues from respiratory failure. In the majority of animals with the atypical form, no blood changes are observed.

Thus, the pathology of stachybotryotoxicosis presents a characteristic set of lesions, including profuse hemorrhage and necrosis in such varied tissues as skeletal muscle, subcutaneous tissues, serous and mucous membranes, and several parenchymatous organs. Hemorrhages occur in the diaphragm, mesentery, large intestine, lymphatic nodes, lung, liver, brain, spinal cord, and adrenal glands. In stachybotryotoxicosis, unlike other diseases causing necrosis of the mucous membranes, the necrotic focal areas are not surrounded by reactive cells; the afflicted animal appears to be incapable of providing inflammatory reactive cells which form a zone

demarcating the lesions.

The signs, symptoms, and lesions in other farm animals generally resemble those described in the equine disease *(18)*.

Russian investigators also described stachybotryotoxicosis in man; where the disease is enzootic in the horse, it frequently occurs in human populations. Several cases have been described in which patients used contaminated straw for fuel or for sleeping mattresses. As an occupational malady, the disease afflicted people who were exposed to dust aerosols heavily laden with a variety of mold spores. These occupations comprised work in cottonseed oil processing plants, grain elevators, textile mills, and in plants processing various grains including the malt of breweries. In all reported cases, a rapid recovery ensued after the individuals were removed from the source of toxins. Reexposure to toxins caused much more serious sequelae in humans than in animals.

Human patients generally develop a dermatitis which usually involves the scrotal and axillary regions; sometimes, however, hands and other parts of the body are also afflicted. The dermatitis leads initially to hyperemia, then to serum exudation, encrustations, and necrosis. In addition to dermatitis, the patients develop catarrhal angina, bloody rhinitis, cough, and pains in throat and chest. Leukocytosis is followed by leukopenia with a sharp drop in white cell counts occurring in the majority of patients.

The toxin produced experimentally by pathogenic strains of *Stachybotrys* is formed in media within 10 days and attains its highest level in about 20 days *(18)*; subsequently, the toxin concentration falls but toxin is still detectable after more than 400 days. The toxin is soluble in various organic fat solvents; anhydrous ethyl ether appears to be the best one. It has been suggested that ingested toxin reacts with gastric juice and is absorbed in a water-soluble form. The material can be extracted from infected straw within about 3 hr after exposure to the mold. Russian investigators claim *(17)* to have isolated the crystalline toxin deciphering an empirical formula of $C_{25}H_{34}O_6$. They suggest that the toxin is representative of a newly discovered group of naturally occurring cardiac toxins, but the precise chemical structure has not been reported. The material is resistent to sunlight, ultraviolet light, and x-rays; it is thermostable and withstands temperatures of 120°C for at least 1 hr. It is unaffected by 2% concentrations of inorganic or organic acids but is readily destroyed by alkaline materials.

Based on these findings, Soviet scientists used sodium, potassium, calcium, and ammonium hydroxide, as well as ammonia and chlorine gas, to detoxify roughage intended for animal consumption.

Although members of the genus *Stachybotrys* have been isolated in almost all areas of the world where it has been sought, there have been no documented cases of toxic disease associated with it in the United States. We should bear in mind, however, that these fungi are present in the United States and that, under proper conditions, they have created public health hazards in other parts of the world.

Penicillium Toxins

Yellowed-Rice Toxins

Shortly after World War II, mold metabolites capable of inducing liver tumors in animals were found by the Japanese in domestic rice imported from Spain, Egypt, Thailand, Burma, Italy, and the United States *(22)*. Several shipments were contaminated with a strain of *Penicillium islandicum* Sopp, the metabolites of which proved to be highly toxic, with liver damage as the major manifestation.

Although more than 15 kinds of fungi have been incriminated in moldy or yellowed rice, this review covers only the most important or the best know types: *P. islandicum* Sopp, *Penicillium citrinum* Thom, and *Penicillium citreoviride* Biourge *(Penicillium toxicarium Miyake)*.

The Japanese isolated *P. islandicum* Sopp in 1948 *(23)*. Tsunoda observed postnecrotic cirrhosis of the liver in rats fed for one month or less on rice contaminated with *P. islandicum*. Voluminous literature recounts the work that has since been done with *P. islandicum* by chemists, pathologists, clinicians, pharmacologists, mycologists, and others *(24)*. Oral administration of a methanol extract taken from the fungus mat which had been cultured on Czapek solution for 14 days induced in mice severe liver damage, mainly centrolobular necrosis and fatty degeneration. Further investigations revealed that the mold metabolites caused chronic

liver damage including cirrhosis and tumors, in rats as well as mice; the end result depended on the amount of moldy rice which had been consumed. According to severity of intoxication, the liver lesions were categorized as acute, subacute, and chronic *(25, 26)*.

Acute intoxication with atrophy of the liver is caused by high levels of toxin given over a short period of time. Animals fed these high levels become inactive, progressively lose both muscular and cutaneous tone, and finally die after a prolonged comatose state similar to hepatic coma in man. Clinical pathology studies reveal several signs of liver damage. In human patients, histopathologic studies show mainly fatty degeneration and hemorrhagic centrolobular necrosis of the liver.

The subacute and subchronic intoxications, induced by lower concentrations of mold toxins over a longer period of time, cause moderate centrolobular necrosis with subsequent collapse of the stroma. These processes lead to fibrosis, liver atrophy, and proliferation of epithelial cells lining bile ducts in the periportal regions. If the animal survives for a few weeks, the liver may show signs of regeneration.

Chronic intoxication develops in mice fed small to medium doses of moldy rice or mold metabolites. These mice survive the early stage of intoxication without showing any signs or symptoms; they usually have a life span of 6 months or more. Post-mortem examination yields a wide range of liver damage from cirrhosis and cancer to slight fibrosis and cell pleomorphism. As with most other toxic conditions, a broad spectrum of clinical and histopathologic responses is observed.

The liver tumors have been described by Saito et al. *(26)* and by Enomoto *(27)*. Histologically, the changes range from mild parenchymal cell hyperplasia to differentiated and undifferentiated liver cell carcinoma. Although there is a high incidence of liver injury, the incidence of malignant parenchymal tumors is relatively low, indicating a low carcinogenic potential for metabolites of *P. islandicum* Sopp.

Among laboratory animals, rabbits were the most susceptible species in regard to metabolites of *P. islandicum* Sopp *(24)*; when fed 1–5% moldy rice, they died within a few days. Those that survived for longer periods developed postnecrotic cirrhosis as soon as 90 days after the initiation of feeding studies. Although rhesus monkeys also showed acute toxic damage of the liver, they developed neither cirrhosis nor tumors as end results of intoxication with metabolites of *P. islandicum*.

As shown by several investigators *(24)*, diet profoundly affects the responses of animals to toxins of *P. islandicum*. Both male and female mice develop acute toxic effects in a short period of time when fed rice infected with *P. islandicum*, but the response is greatly enhanced by low protein intake, as illustrated by the following example. The death rate of mice during three weeks of feeding with an 11% protein diet containing 3% moldy rice was about 44% in males and about 25% in females. The death rate of mice fed the same percentage of moldy material with a 34% protein diet was 28% in males and 5% in females. As toward many other liver toxins and carcinogens, the male of the species was considerably more sensitive.

Organs other than the liver are also injured by exposure to toxic metabolites of *P. islandicum (28, 29)*. The pathological findings include atrophy of the thymus, spleen, and fat tissues. Fatty degeneration of the tubular epithelium of the kidney occurs, and pancreatic cirrhosis occasionally develops in mice and rats. In addition, various tumors originate in tissues other than the liver.

One of the toxic agents isolated from the media in which *P. islandicum* Sopp was grown received the name luteoskyrin *(30, 31)*. The material was obtained from the media as one of seven pigments, including rugulosin and cyclochlorotine *(24)*. To isolate pure luteoskyrin from culture medium, a relatively simple method was described by Tatsuno *(32)*.

Several physical and chemical properties of luteoskyrin VI and its related compound, rugulosin (V) are compiled in Table 3. The chemical structure of luteoskyrin was determined with data from color tests, ultraviolet absorption spectra, infrared absorption spectra, chemical reactions, NMR spectra, and x-ray diffraction. These various tests were previously described *(24)*.

One outstanding characteristic of luteoskyrin is its extreme sensitivity to sunlight, leading to photochemical changes of luteoskyrin in several different organic solvents. The product of photodecomposition is lumiluteoskyrin.

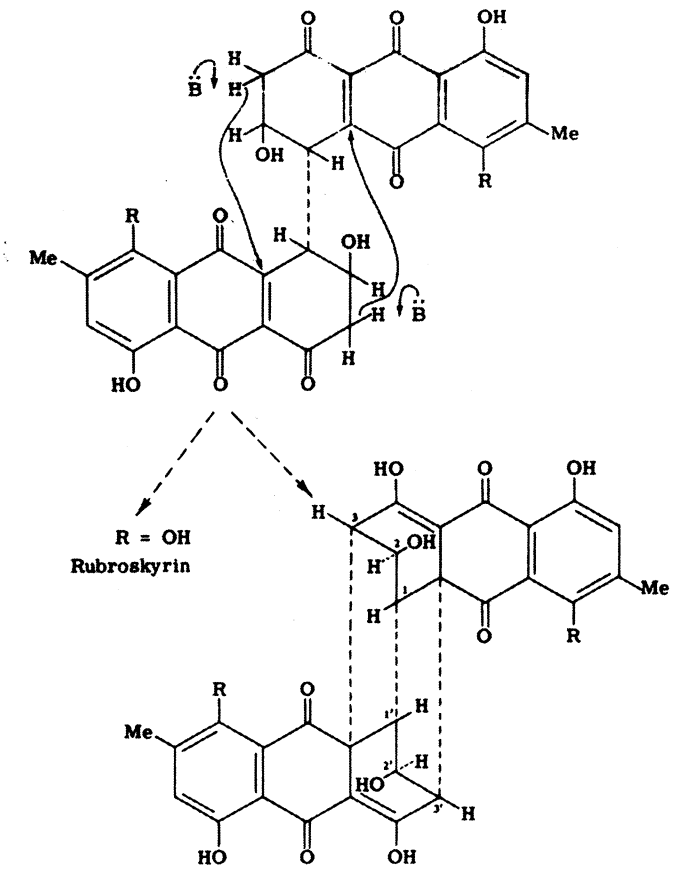


Table 3. Physiochemical properties of luteoskyrin and rugulosin.

Property	Luteoskyrin	Rugulosin
Molecular formula	$C_{30}H_{22}O_{12}$	$C_{30}H_{20}O_{10}$
Molecular weight	574	542
Melting point, °C	287 (dec)	290 (dec)
$[\alpha]_D^{25}$	−880 (acetone)	+492 (dioxane)
Infrared (Nujol), cm^{-1}		
CO	1623	1690, 1620
OH	3378	3450

[a] Data of Saito et al. *(24)*.

Luteoskyrin appears to have antimicrobial activities against various microorganisms, and this characteristic has made possible a bioassay based on the sensitivity of a mutant of *Escherichia coli* Q-11.

As revealed by extensive studies *(23)*, the biosynthesis of luteoskyrin depends on environmental factors. For instance, toxicity tends to decrease when the mold is grown on various grains in the following order: rice, barley, wheat, and corn. The addition of certain amino acids to the culture medium, including asparagine, glutamine, and malonate, appears to increase the production of luteoskyrin and of other pigments as well. As shown by labeling studies, these amino acids are incorporated into the luteoskyrin molecule and into the chemical structure of the related pigments *(33)*. Luteoskyrin, rugulosin, and their associated pigments are produced under similar circumstances, but the two series of compounds are derived along pathways which diverge at an early stage of biosynthesis.

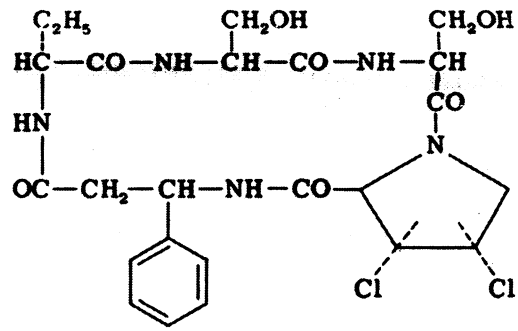
The biological effects of luteoskyrin include a swelling of the mitochondria and an inhibition of oxygen uptake by homogenates of rat liver, kidney, and heart muscle. The major site of inhibition apparently involves the mitochondrial phosphorylation reaction. Luteoskyrin binds to DNA *in vitro*. Studies on liver, microsomal preparations, and supernatant indicate that the toxin evokes a high, prolonged incorporation of cystine into the liver which impairs the transfer of sulfur-containing amino acids into protein. Thus, to varying degrees and by different mechanisms, luteoskyrin apparently damages several intracellular granules.

Toxicity of luteoskyrin varies with the route of administration *(24)*. In mice, the LD_{50} (mg/kg body weight) is as follows: intravenous, 6.65; intraperitoneal, 40.8; subcutaneous, 147; and oral, 221. Repeated subcutaneous injection of less than 1/10 of the LD_{50} over several days produces the same lethal effect as a single subcutaneous LD_{50} but requires a longer time. Very young mice are more sensitive than older ones, and males are more sensitive than females.

The pathologic effect of luteoskyrin resides primarily in the liver, being similar in rats, mice, rabbits, and monkeys. The microscopic changes of yellow discolored liver can be seen within 24 hr after exposure. Marked centrolobular necrosis and fatty degeneration occur, with some nuclear pleomorphism and hyperchromatosis. Following prolonged exposure, mice develop liver tumors but not cirrhosis.

The pathologic response to rugulosin reportedly is almost identical with that to luteoskyrin.

Besides luteoskyrin, rugulosin, and associated pigments, a chlorine-containing peptide and another toxin, referred to as islandotoxin, have been isolated from the culture filtrate of *P. islandicum*. The chlorine-containing peptide is termed cyclochlorotine; its structure is unknown; its empirical formula is $C_{25}H_{36}N_5O_8Cl_2$, and its melting point is 251°C. The chemical structure VII has been suggested for islandotoxin, but the precise structure is still not clearly established. Although both chlorine-containing peptide and islandotoxin share certain characteristics, they apparently are not identical.



VII

Cyclochlorotine, a rapidly acting hepatotoxin, causes the disappearance of membrane-bound ribosomes and glycogen granules from injured liver cells within a very short time. Thus it disturbs both protein and carbohydrate metabolisms. In mice, it causes an initial hyperglycemia followed by hypoglycemia; it also causes a disturbance in the number of liver enzymes which accelerates glycogen catabolism and inhibits glycogen neogenesis.

Pathologic effects of cyclochlorotine are largely restricted to the liver comprising an interference with circulation and an increased permeability of the capillaries. (When the peptide is introduced into the skin of dogs, a similar vascular effect occurs locally and leads to necrosis of the epidermis and leukocytic infiltration.) In addition, vacuolation and hyaline droplet formation occur in the parenchymal cells, particularly in the perilobular areas. Acute toxicity produces also a proliferation of the smooth endoplasmic reticulum.

Chronic exposure of mice to the peptide results in cirrhosis and liver cell carcinoma along with tumors of the reticuloendothelial system. Rats develop peritoneal hemorrhage as the result of acute pancreatic necrosis; these complications, however, usually occur only after long-term exposure.

Although the toxins luteoskyrin, islandotoxin, and cyclochlorotine share many pathological effects, their biological effects are quite different. Luteoskyrin and islandotoxin cause liver damage characterized by centrolobular necrosis. Cyclochlorotine, on the other hand, causes damage in the peripheral zone, characterized by vacuolation of liver and endothelial cells and appearance of hyaline

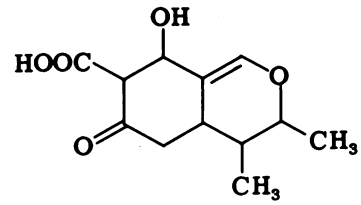
droplets in the cytoplasm. A similar cytotoxic effect results from cysteine deficiency or from allyl formate administration.

Long-term feeding studies with cyclochlorotine and luteoskyrin in mice have shown that luteoskyrin has a hepatotoxic as well as a possible carcinogenic action, but it is less potent than aflatoxin. The chlorine-containing peptide is a cirrhogenic agent and may be carcinogenic, although this aspect requires further study; in limited experiments, a few mice have developed liver tumors.

An additional toxin isolated from *P. islandicum* Sopp has been designated erythroskyrine *(34);* it has not yet been as well characterized as the already discussed toxins.

In 1953, Japanese investigators discovered that some rice imported from Thailand was contaminated with *Penicillium citrinum* Thom, a producer of potent toxins *(22).* The mold has since been found in all rice-producing areas of the world, including Japan, Burma, Italy, Egypt, the United States, and more recently in the People's Republic of China. Mice and dogs fed rice contaminated with *P. citrinum* displayed enlarged kidneys which, on histopathologic examination, revealed marked degeneration and dilatation of the lower nephrons beneath Henle's loop *(24, 35).* A flattening and desquamation of tubular epithelium obstructed the lumen at the corticomedullary junction, partially accounting for the renal lesion. More prolonged feeding of infested rice to mice resulted in glomerular lesions such as adhesion of the tuft to the capsule. The renal lesions resembled glomerulonephrosis, a picture often seen in toxic nephrosis.

Citrinin is the major yellowed-rice toxin produced by *P. citrinum;* its chemical structure (VIII) has been established *(36, 37)* and its biosynthesis has been partially determined.



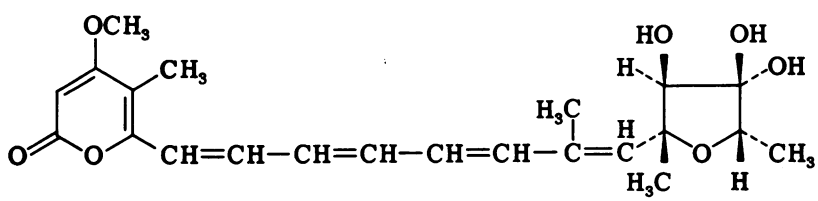
VIII

The LD_{50} of citrinin has been reported for mice, rats, rabbits, and guinea pigs; the subcutaneous LD_{50} varies among these species from 35 to 67 mg/kg body weight. Intraperitoneally, the LD_{50} is 30 mg/kg in mice and about 50 mg/kg in rabbits.

Besides renal damage, citrinin causes acetylcholine or pilocarpine-like responses, including vasodilation, constriction of the bronchi, and increased muscular tone.

One additional mold associated with yellowed rice was isolated several years ago from rice collected in Taiwan and Japan. Designated as *Penicillium toxicarium* Miyake, it was later shown to be identical with *Penicillium citreoviride* Biourge, which had been described earlier by two laboratories *(24).* Feeding studies proved the contaminated rice to be toxic to rats. Rice infested with *P. citreoviride* was studied for its toxicologic effects; more recently, an active fraction has been isolated, chemically identified *(24, 38),* and designated as citreoviridin (IX).

The toxicology of citreoviridin is known to a limited extent for several mammalian species. It causes a typical acute poisoning that is characterized by an early onset of progressive paralysis in the hindlegs, vomiting, convulsions, and respiratory disorder. At an advanced stage, cardiovascular disturbances, flaccid paralysis, and hypothermia occur along with dyspnea, gasping, and coma; respiratory arrest and death



IX

follow. The symptoms in animals resemble closely those in human patients suffering from acute cardiac beriberi—also called shoshin-kakke—a common disease throughout Asian countries in the past. The cause of this type of beriberi remains unknown; there appears to be ample thiamine in the tissues of patients. In both man and animals, the disease is characterized primarily by ascending progressive paralysis.

Despite the very severe clinical symptoms attributable to citreoviridin, histopathological changes are minimal or not detectable at all.

The Rubratoxins

The rubratoxins are a group of metabolites produced by *Penicillium rubrum* Stoll. Growing on feeds, some strains produce large quantities of rubratoxins which create an actual hazard to livestock and a potential hazard to man *(39)*. The mold grows on a large variety of feedstuffs, and during the past decade some information on the growth and toxicity of crude compounds has accumulated.

In the earliest accounts describing a disease in cattle and swine, Sippel et al. *(40)* isolated 13 different molds from toxin corn. Only two of these, *A. flavus* and *P. rubrum* caused illness and death when fed to laboratory experimental animals. Further studies showed that the isolate of *P. rubrum* was considerably more toxic than that of *A. flavus*. When fed to pigs over a period of about 5 days, a total dose of 7–8 lb of corn contaminated with *A. flavus* resulted in death; however, a single dose of only ½ lb of corn contaminated with *P. rubrum* resulted in death, usually within 24 hr.

Burnside et al. *(41)* reported moldy corn poisoning in cattle; they isolated two organisms, *Aspergillus flavus* Link and *P. rubrum* Stoll, from corn associated with the disease. When grown in proper media, these cultures produced a material that was lethal to mice, horses, pigs, and chick embryos.

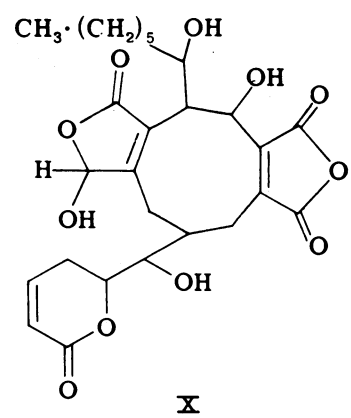
Although *P. rubrum* is probably the most prominent mold associated with this type of toxicosis, other genera, including *Fusaria* and *Aspergilli*, produce toxins when grown on corn products; in fact, the naturally occurring disease probably results more often than not from the interaction of several toxins produced by different molds. Seibold and Bailey *(42)* described a form of toxicosis in dogs, designated hepatitis X, in the early 1950s. Following detailed experimental studies, they concluded that hepatitis X in dogs and moldy corn toxicosis in pigs shared the same etiology.

After aflatoxins were discovered and chemically identified in the 1960s, it was realized that they acted synergistically with the toxins produced by *P. rubrum;* although aflatoxin alone induced many of the manifestations observed in field cases of hepatitis X, only the synergistic action of aflatoxin B_1 and rubratoxin B provoked the full spectrum of manifestations characterizing the disease *(43)*.

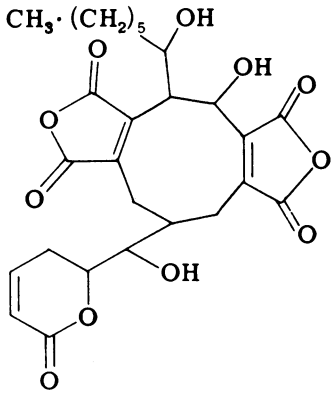
Forgacs and Carll *(44)* described a hemorrhagic disease related to contaminated poultry feed. They subsequently demonstrated that *P. rubrum* and the closely related species *Penicillium purpurogenum* caused good grain to become toxic to chicks. With the toxic feed, they reproduced the hemorrhagic disease seen in field cases.

Isolation of the two principal toxins, rubratoxin A and rubratoxin B, was achieved by investigators who worked with a partially purified substance that was produced by molds grown in pure culture. The toxins have been chemically characterized and their structures proposed (X and XI) *(45–48)*.

Rubratoxin B (XI) is clearly the principal toxin produced by toxigenic strains of *P. rubrum*, but there is still very little known about its distribution in feeds and foods. Furthermore, except for the report of Wogan et al. *(49)*, little information is available on toxicity of the purified compounds studied under controlled conditions.



X



XI

Although it is difficult to assess the role of *P. rubrum* in naturally occurring diseases, there is little doubt that strains of this mold can produce large quantities of toxic metabolites under laboratory conditions and that the mold is often isolated from feeds collected during outbreaks of disease in animals. The magnitude of the problem remains to be elucidated.

Both growth and toxin production of *P. rubrum* vary considerably, apparently depending upon the composition of the media upon which it is cultured. Complex substrates support considerably greater growth and toxin production than do synthetic media.

Pure rubratoxins A and B are soluble in acetone, moderately soluble in alcohols and esters, and minimally soluble in water. The two compounds are completely insoluble in nonpoplar solvents. Rubratoxin A (X) is much more soluble in ethyl alcohol, however, than is rubratoxin B; conversely, rubratoxin B is by far the more soluble in ethyl acetate. These characteristics have been used to separate the compounds individually from the culture medium. Thin-layer chromatography is useful for this purpose. From mixtures of benzene and ethyl acetate, pure rubratoxin B crystallizes as long lathes with the geometry of hexagonal plates extended along one axis in the plane of the hexagon. In diethyl ether, pure rubratoxin B crystallizes as rosettes. It may crystallize as regular hexagonal plates, however, from solvents such as amyl acetate. The mass spectra of both rubratoxin A and B are known, as are the ultraviolet, infrared, and other useful analytical spectra.

Forgacs and Carll *(50)* described hemorrhage and congestion of the breast, lung, kidney, spleen, and many other organs and tissues of chicks feeding on grain contaminated with *P. purpurogenum* and *P. rubrum*. Extracts from *P. rubrum* grown on cracked corn were lethal to guinea pigs, mice, rabbits, and dogs. According to Wilson and Wilson *(45)*, the manifestations of the disease closely resembled those described by other investigators using moldy feeds.

Sprague-Dawley rats injected with a sublethal dose of crude rubratoxin developed a severe fatty infiltration of the liver *(51)*. Despite visible damage to the mitochondria, no significant impairment of mitochondrial function was shown by *in vitro* tests. Townsend et al. *(46)* gave rubratoxins to mice in doses so small that microscopic lesions were absent. Nonetheless, metabolic processes in the livers of these mice were adversely affected; after a standard dose of phenobarbital, the sleeping time was increased.

The liver is clearly the major site of injury inflicted by rubratoxins; this is not surprising because the liver plays a central role in detoxification.

Recently, Wogan et al. *(49)* investigated the acute and subacute toxicities of rubratoxin B; they administered the material by various routes to several species of animals. They demonstrated an interaction between rubratoxin B and aflatoxin B_1. In all of the tested species, the most important lesion was in the liver, comprising congestion, hemorrhagic degeneration, and necrosis. There was also general depletion of lymphocytes in the spleen and the lymph nodes along with frequent hemorrhages at these sites. Of the various species, cats were the most sensitive, developing hemorrhages and massive ascites in addition to the lesions. Following a sublethal dose of rubratoxin, the livers of guinea pigs, ducklings, mice, and rats regenerated over a period of 7 days. Occasionally, mild renal damage was observed microscopically, but the finding was not consistent among the various species or within one particular species.

Chronic toxicity studies yielded a considerable body of important information *(49)*. Very low doses of rubratoxin B administered over long periods of time were not carcinogenic, although they caused severe liver damage (Tables 4 and 5). Given a maximum tolerated level of rubratoxin B, none of the 60-week survivors showed any evidence of neoplastic or even

Table 4. Lethal potency of rubratoxin B in several species.[a]

Species	Sex	Weight, g	No. of animals	Dosing route[b]	Vehicle[c]	LD_{50}, mg/kg
Rat	M	58	60	IP	PG	0.36 (0.27–0.49)[d]
Rat	F	60	50	IP	PG	0.36 (0.28–0.46)
Rat	F	59	70	IP	DMSO	0.35 (0.28–0.45)
Rat	M	60	25	PO[e]	DMSO	ca. 400
Rat	F	58	25	PO	DMSO	ca. 450
Mouse	F	25	25	IP	DMSO	0.27 (0.22–0.34)
Mouse	F	25	30	IP	PG	2.6 (2.0–3.1)
Guinea pig	M	565	18	IP	DMSO	0.48 (0.41–0.56)
Cat	M	3000	3	IP	DMSO	ca. 0.2
Cat	M, F	3000	8	IP	PG	1.0–1.5
Dog	M	3000	7	IP	PG	>5.0
Chicken	M	500	6	IP	PG	>4.0

[a] Data of Wogan et al. *(49)*.
[b] Dosing routes: IP, intraperitoneal; PO, by mouth.
[c] Vehicles: PG, propylene glycol; DMSO, dimethyl sulfoxide
[d] 95% confidence interval.
[e] By stomach tube.

Table 5. Toxicity in male rats exposed simultaneously to rubratoxin B and aflatoxin B_1.[a]

Treatment regimen	Total toxin, mg/rat	Body weight at end of dosing, % of control	Mortality	No. of preneoplastic liver lesions at 70–80 wks
Controls (DMSO 3×/wk for 5 wk)	—	100	0/10	0/7
Rubratoxin B (25 mg/kg, 3×/wk for 5 wk)	39.7	95	0/10	0/7
Aflatoxin B_1 (0.2 ppm in diet for 6 wk)	0.11	102	0/10	6/7
Rubratoxin B and aflatoxin B_1 simultaneously	—	86	9/20	5/8

[a] Data of Wogan et al. *(49)*.
[b] Data of Newberne and Wogan *(52)*.

preneoplastic lesions; several animals survived as long as 87 weeks without any evidence of neoplasia.

The synergism between rubratoxin B and aflatoxin is important from the standpoint of public health. Rubratoxins, while not particularly toxic by themselves, may well be potentiating factors for other toxins simultaneously occurring in moldy feeds and foods. The interaction of the more prominent mycotoxins must be examined in detail to determine their hazard to public health.

Other Penicillium Toxins

Other *Penicillium* toxins comprise patulin, penicillic acid, and certain lactones; they are associated with several species of *Penicillia* and *Aspergilli*, including *Penicillium urticae*, *Penicillium claviforme*, *Penicillium expansum*, *Aspergillus clavatus*, *Aspergillus giganteus*, and *Aspergillus terreus*. Although not studied as extensively as others, the toxins induce significant biological effects. In mice, they reduce the lymphocyte count in blood, increase vascular permeability (resulting in edema), and suppress the formation of urine. They also elevate the blood sugar level. Patulin has been used therapeutically in very low concentrations as a nose and throat spray and as a treatment for common head cold.

The molds and their metabolic products were described in detail by Ciegler et al. *(53)*.

An additional class of compounds, cyclopiazonic acid and related toxins, are produced mainly by *Penicillium cyclopium* Westling. This mold and its toxic metabolites have been isolated from peanuts and corn meal. The biologic effects of cyclopiazonic acid are only partially known. When the toxin is given orally to ducks, chicks, and rats, the animals display convulsions and die within a very short time. Other but less well-defined toxins are also associated with *P. cyclopium* Westling.

Miscellaneous *Penicillium* toxins were described by Wilson *(54)*; although some of the toxic fractions have been isolated, they are generally not yet sufficiently characterized for inclusion in the discussion.

Aspergillis Toxins (Aflatoxins)

Although *Aspergillus flavus* is associated with most food and feed contaminations, only a few strains of *A. flavus* actually produce aflatoxins; however, these few strains are the most important producers of aflatoxin. Other *Aspergilli*, too, produce aflatoxins *(55)*.

Taber and Schroeder *(56)* found that *Aspergillus flavus-oryzae*, isolated from peanuts grown in the United States, produced no aflatoxin G_1 or G_2, although several cultures produced B_1. Likewise, the aflatoxins originating from *Penicillia* (e.g., *Penicillium citrinum*, *Penicillium frequentans*, *Penicillium variable*, and *Penicillium puberlum)* were considered a relatively minor hazard *(57)*.

Aspergillus toxins other than aflatoxin were reviewed previously *(57–63)*.

Early Studies

The majority of the more than 1000 publications about aflatoxins appeared during the last 10 or 12 years, reflecting an intense interest in mycotoxins, and more specifically in aflatoxins, which was aroused by the outbreak of turkey X disease throughout England in 1960 *(64)*.

During the early development of antibiotics, a fundamental discovery was made that some materials from antibiotic-producing molds were toxic in animal trials. However, these molds were usually discarded because they were poor producers of antibiotics; little attention was paid to their potential for producing toxins. Thus, the recognition of mycotoxins as a threat to public health was delayed for two or three decades.

It has long been known that fungi growing on foods and feeds are ubiquitous; they may contaminate virtually every stable foodstuff of either plant or animal origin. The majority grows throughout extremely wide ranges of pH and temperature, making moisture conditions the primary environmental restriction. On cereal grains and oil seeds, molds grow at moisture levels which are commonly encountered under storage conditions. These storage molds mainly are species of *Aspergilli* and *Penicillia*. For instance, moldy corn toxicosis in swine, occurring with considerable frequency in the humid southeastern parts of the United States, is associated with both *Aspergillus* and *Penicillium* molds *(41, 65)*.

The small number of publications indicated

clearly the lack of interest in mycotoxin research prior to the 1960 outbreak of turkey X disease. Several of the early reports *(40, 66)* associated liver disease in swine with a ration that contained corn or peanuts. *Aspergillus* molds were incriminated in the epizootics of bovine hyperkeratosis, although later studies indicated that the disease was more complex; it probably resulted from an interaction of chlorinated cyclic hydrocarbons with mold toxins *(67–69)*. Forgacs et al. *(44, 50)* isolated several molds, most species of *Aspergilli* and *Penicillia*, from feeds that led to a hemorrhagic disease in poultry. Furthermore, isolates from these molds on poultry feed were often capable of reproducing the hemorrhagic syndrome in birds. These diseases were then designated as moldy feed toxicoses. The English episode in turkeys brought together microbiologists and veterinarians who recognized that toxic manifestations were caused by the ingestion of certain mold-contaminated feeds.

During the period from 1960 to 1962, several later reports *(70–72)* about outbreaks of disease in poultry and fish at diverse geographical locations incriminated fungi in feeds as the likely etiologic agents. In avian species, the acute disease resulted in loss of appetite, weakness of wings, and lethargy. Histologic examination of tissues revealed an acute hepatic necrosis and usually a marked bile duct proliferation. Ultimately, the problem was traced to imported Brazilian peanut meal in the birds' rations. It was then discovered that ducklings, swine, and cattle had also been poisoned by similarly contaminated lots of peanut meal. The Brazilian peanut meal, however, was not alone in causing toxicity. At about the time when the turkey X disease broke out, a similar disease, associated with peanuts processed in East Africa, affected ducklings in Kenya *(73)*. The early discovery that ducklings were particularly sensitive to aflatoxin, as evidenced by a rapid and extensive bile duct proliferation, was fortuitous; this species became the major tool for biologic assay. Following the initial studies of peanut meal poisoning in turkeys and ducklings, Lancaster et al. *(74)* reported that rats developed liver cell carcinomas when toxic peanut meal was included in their diet for 30 weeks or longer. Sargeant et al. *(75)* and Nesbitt et al. *(76)* identified the toxin-producing organism in the peanut meal as the saprophytic mold *Aspergillus flavus* Link ex Fries, a widely distributed organism that has been isolated from virtually every staple food product in the tropical and semitropical areas of the world *(77)*.

While the turkey X disease was at its height, an epizootic of liver cancer afflicted hatchery-reared rainbow trout in Washington and Oregon *(72, 78)*. Investigations then revealed that liver carcinoma occurred in fish at many different locations. Trout raised in hatcheries had for many years been fed a diet including vegetable sources of protein, with cottonseed meal as the major source *(79)*. As a result of many investigations, the etiology of trout hepatomas was finally traced to contaminants in the cottonseed meal. Furthermore, the investigators discovered the influence of nutritional factors on both the neoplastic processes *(80)* and the resistance to carcinogenic toxins *(81)*.

In addition to toxicoses in various domestic animals and liver carcinomas in trout, an observation in laboratory rats further heightened the interest in contaminated feed. During studies comparing the effects of choline-deficient and adequate diets, Salmon and Newberne *(82)* observed liver carcinoma in rats were fed diets containing peanut meal. Within a relatively short time, several reports *(83, 84)* showed that the contaminant in peanut meal was the same as that produced by a strain of *Aspergillus flavus* which was isolated from toxic meal in England and identified as a complex of metabolites *(73, 85, 86)*.

Identification and Characterization of Aflatoxins

The discovery that the toxins had a characteristic fluorescence pattern on thin-layer chromatograms greatly facilitated their isolation and characterization, culminating in the determination of the molecular formulas of four components designated as aflatoxins B_1, B_2, G_1, and G_2; the four components were distinguished by their blue or green fluorescence and by their R_f values on thin-layer chromatograms *(87)*. More recently, related substances have been isolated and chemically characterized, the most important ones being M_1, M_2, B_2, and G_{2a} *(63)*. The M_1 and M_2 fractions, first isolated from the milk of cows feeding on aflatoxin-contaminated fodder *(88)* were later found in the milk of laboratory rats. M_1 and M_2 possessed the same

toxicity as the aflatoxins from which they were derived *(89)*. On the other hand, the hydroxylated B_{2a} and G_{2a} derivatives of B_2 and G_2 described by Dutton and Heathcote *(90)* were virtually nontoxic, suggesting that contaminated foods may be detoxified by acid treatment.

The isolation, purification, and identification of the various fractions of aflatoxins are described in detail by several authors *62, 91–93*). The structures are shown in Figure 1.

Although not highly specific, quantitative assays for aflatoxins are reasonably accurate. The current methodology relies on thin-layer chromatography, the use of known reference materials, derivative formation, and bioassay. The same methodology, however, does not work satisfactorily for all food products. For example, corn and corn products require modifications of the methods that are commonly used to assay for aflatoxin in cottonseed, cocoa, oats, and peanuts *(94)*.

A biological test, using the duckling, supplements the various chemical methods for analysis *(84)*. One-day-old ducklings are usually used, and the suspected toxin is dissolved in either proplylene glycol, dimethyl sulfoxide, or some other suitable solvent. The ducklings are then dosed with varying amounts of the material, killed within 48–72 hr, and a section of each liver is examined for characteristic histologic alterations (Figure 2). The alterations include periportal liver cell necrosis and bile duct hyperplasis, the latter being graded to mirror the concentration of administered aflatoxin (Table 6). The histologic changes are described by Newberne et al. *(84)* and the relative toxicities of the compounds are listed by several investigators *(95, 96)*. The bile duct hyperplasia is not specific for the toxins, but

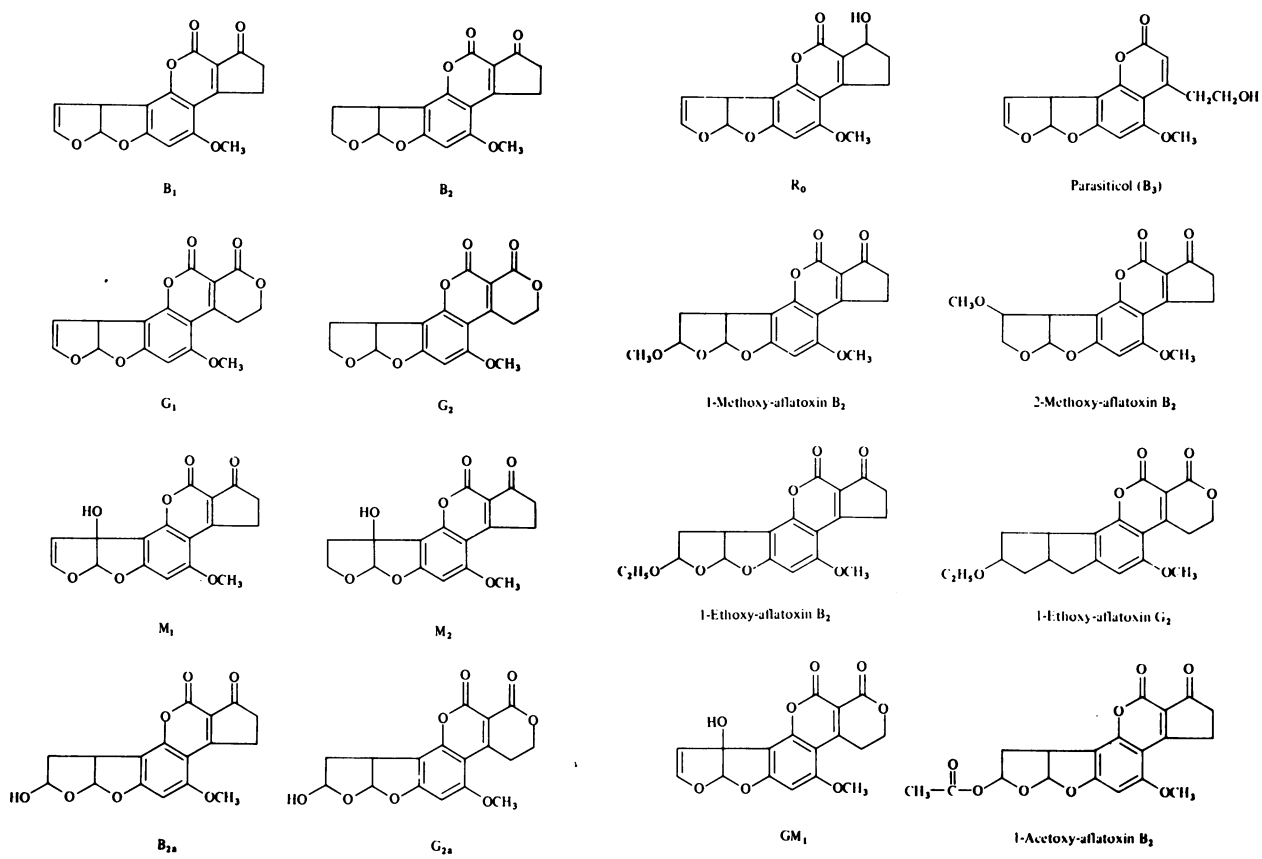


FIGURE 1. Chemical structures of aflatoxins and metabolites.

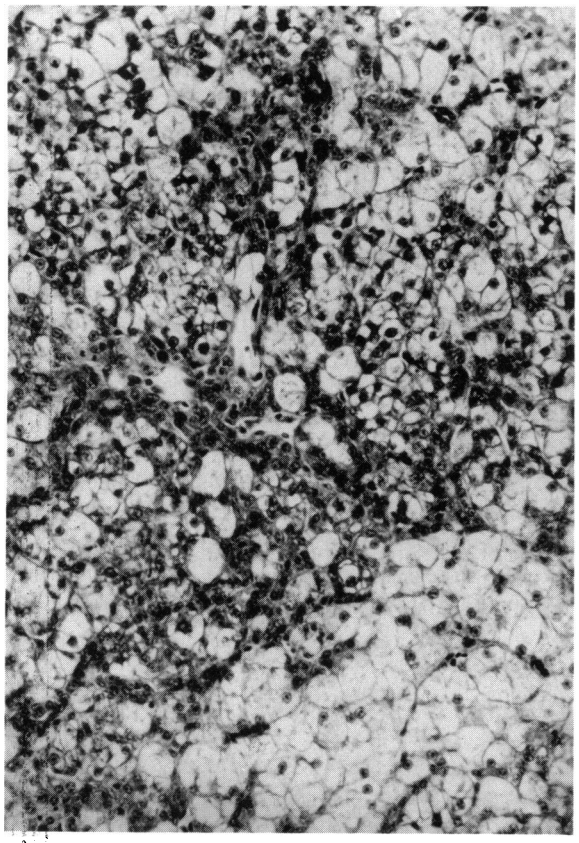


FIGURE 2. Typical bile duct hyperplasia in duckling liver following exposure to aflatoxin.

provides a reliable confirmation of the results obtained with chemical isolation and thin-layer chromatography.

The biosynthesis of aflatoxins has been studied extensively. In the earlier work *(97)*, various precursors were labeled and tested for incorporation into the synthetic pattern of aflatoxin B_1. Phenylalanine and shikimic acid appeared implicated as precursors *(98)*. Although several hypothetical schemes were devised, the precise nature of the biosynthesis remains to be established. On the other hand, procedures for laboratory synthesis of aflatoxins B_1, G_1, and M_1 have been developed *(99)*; the syntheses were designed primarily for verification of the structure; the procedures were too complex to furnish a routine production of material for experimental use.

A total of 12 structurally related compounds with similar configurations have been identified; Buchi and Rae *(100)* described in detail both the structure and chemistry of many of these compounds. Products of metabolism in the biological systems also were investigated; Wogan *(63)* recently reviewed the chemistry involved in these processes.

Table 6. Effects of toxic materials on ducklings in 7 days. [a]

Dietary supplement	Initial weight, g	7-Day weight, g	Liver weight		Average bile duct hyperplasia [b]
			Total, g	% of body weight	
None	57	—	— [c]	—	38.0
None	55	144	5.5	4.7	30.0
None	47	170	11.8	6.3	0
500 μg liquid culture extract	58	127	7.4	5.8	6.0
7.8 μg wheat culture extract	54	138	9.7	5.0	5.0
2.0 μg pure aflatoxin B_1	51	118	6.0	5.0	10.0
15.6 μg pure aflatoxin G_1	54	121	5.4	4.4	10.0
50.0 μg pure aflatoxin B_2	57	147	7.1	4.9	16.0

[a] Data of Newberne *(84)*.
[b] Graded 0 to 4+. multiplied by 10, and averaged.
[c] Died before 7 days.

Effects of Aflatoxin on Various Species

Mollusk Eggs: Various biological assay methods for aflatoxins have been proposed with the goal of increasing sensitivity and speed of detection *(101)*. Townsley and Lee *(102)* reported that aflatoxin B_1 inhibits cell cleavage in fertilized eggs of the mollusk *Bankia setacea* but prevents neither fertilization nor nuclear division. It has been suggested that the eggs be used as a bioassay system because their handling requires a minimum of technique and training and they are sensitive to aflatoxin concentrations of 0.05 μg/ml. However, limiting features include the availability of the mollusk, the requirement of sea water, and the need to work at low temperatures.

Embryonated Eggs: Embryonated chicken eggs are also sensitive to aflatoxin, but their use is limited by high death rates *(103, 104)*. The biological effects are interesting, however, consisting of growth retardation, edema, hemorrhage, decreased brain development, skeletal defects, and beak anomalies. Their nonspecific nature diminishes the usefulness for assays.

Tissue Culture: Tissue culture studies indicated that aflatoxin suppressed mitotic division in heteroploid and diploid embryonic lung cells *(105)*. In concentrations from 0.5 to 1.0 ppm, aflatoxin reduced growth of heteroploid embryonic lung cells of human origin *(106)*; at 5.0 ppm, the cells did not grow at all. Nucleolar volume decreased 12 hr after chick embryonic cells were dosed with aflatoxin, and the nucleoli disappeared eventually *(62)*.

Plants: It was reported that aflatoxins caused albinism in the leaves of young cress. *(Lepidium stivum)* that were subjected to aflatoxin concentrations ranging from 1 to 10 μg/ml *(107)*. Seed germination was prevented by higher doses; the authors suggested that the effects could be elaborated into a simple test for the detection of aflatoxin; however, this proposal never was implemented.

Microorganisms: In 1966, Burmeister and Hesseltine *(108)* surveyed 325 microorganisms for sensitivity to aflatoxin. Many of the various organisms (bacteria, fungi, algae, and protozoa) were affected, but the damage was not sufficiently specific to warrant further investigation. Clements *(109)* used similar methods but the sensitivity was not sufficient for a useful test.

Domestic Animals: The carcinogenicity of aflatoxin is well established. Fish, birds, ferrets, trout, pigs, sheep, and laboratory rats develop liver carcinoma under appropriate conditions *(52, 95, 110, 111)*. Although liver cell carcinomas comprise the majority, primary tumors also arise in other organs such as kidney, stomach, lung, salivary and lacrymal glands, colon, and skin.

Although aflatoxin poisoning apparently has long been a problem in animals throughout the world *(112)*, the majority of records and observations appeared after 1960, as shown by the following: Loosmore and Markson *(85)* observed that the clinical signs and liver lesions in cattle suffering from aflatoxin poisoning resembled those produced by the pyrrolizidine alkaloids. The liver lesions produced in cattle by these two different classes of toxins were, in fact, indistinguishable. Furthermore, they resembled lesions observed in children with "childhood cirrhosis." In 1963, Allcroft and Lewis *(113)* reported that calves suffering from aflatoxin poisoning had a decreased rate of growth and severe tenesmus. Post-mortem examinations revealed visceral edema, ascites, and fibrosis of the liver. Similarly, well-controlled experiments with steers showed that aflatoxin significantly decreased weight gains and feed efficiencies over a 133-day trial *(114)*. Given large doses of aflatoxins, milk cows showed decreased milk production and occasional weight loss. When fed aflatoxin, cattle produce milk containing the M_1 and M_2 fractions. In most of the cattle studies, enzymes of serum and liver were affected, but the changes were equivocal. As one important observation, aflatoxin-fed cattle showed a marked decrease in serum vitamin A *(115)*.

Swine are very sensitive to aflatoxins *(116)*; clinical observations of aflatoxicosis in swine have been recorded in detail. As with other species, the age of the animal is important, the younger being more sensitive. The marked liver damage occurs mainly in the centrolobular zone but may involve the entire lobule in cases of exposure to high concentrations *(117)*. Serum enzyme levels are increased, reflecting liver damage; vitamin A concentrations in serum and

liver are markedly decreased. Aflatoxin-induced liver cancer in swine is unknown.

Sheep apparently are least sensitive domestic animals, although they excrete relatively large amounts of aflatoxin metabolites in urine and milk *(118)*. Despite their relative insensitivity, sheep develop liver damage after exposure to high levels of aflatoxins; and if exposure continues over a long period, they develop liver cell carcinoma, nasal carcinoma, and chondroma *(119)*.

The early observations of a toxic disease in dogs in the southeastern United States *(42, 112)* led to the discovery that the majority of toxic episodes were associated with feed containing peanut meal. The disease, referred to as hepatitis X, shared many characteristics with moldy corn poisoning in swine, and investigators finally concluded that there probably was a common etiologic agent. Dogs suffered hemorrhages in various tissues and the liver underwent cell necrosis, severe fatty change, cirrhosis, and fibrosis. Bile duct proliferation varied with the amount and duration of exposure. The disease was reproduced in dogs by aflatoxin B_1 and by a combination of aflatoxin B_1 and rubratoxin B *(43)*.

Poultry are highly susceptible to aflatoxins. Not only is the liver seriously injured, but the kidney also sustains damage *(83)*. Ducklings, quail, turkeys, and chickens (in order of decreasing sensitivity to aflatoxin) respond with bile duct proliferation and various degrees of liver cell damage *(95)*. Fatty liver, common in all species, is most marked in the very young duckling, the condition partly resulting from residue of the yolk. Long-term studies *(65, 120)* reveal that the ducks often develop coexistent liver cell and bile duct carcinomas as a consequence of exposure to aflatoxins for sufficiently long periods. The dose to produce carcinoma is quite low in comparison with carcinogenic doses for other species developing liver tumors.

Monkeys: Madhavan et al. *(121)* presented the pathologic features of lesions produced by aflatoxin in the livers and kidneys of monkeys. The administered doses were so high, however, that the animals did not survive very long; thus, lesions were primarily acute or subacute toxic responses. The major findings were biliary fibrosis associated with severe fatty change and parenchymal necrosis. Determined chemically, liver fat was sharply increased.

Cuthbertson et al. *(122)*, conducting a long-term experiment with cynomologous monkeys, observed that the feeding of aflatoxin B_1 at a level of 2 μg/kg body weight per day had very little influence on the monkeys; but if the dosage was increased to 50 μg/kg body weight daily, the monkeys died in 1 to 2 months after initiation of exposure.

In African monkeys receiving 0.10 to 1.0 mg aflatoxin per day, survival time ranged from 6 to 22 days, generally depending on the dose *(123)*. There was a consistent pattern of toxicity to the liver which was similar to that seen in man.

During an extensive study of rhesus monkeys, Deo et al. *(124)* observed that monkeys receiving a mixed aflatoxin preparation at a level of 1 mg/kg body weight per day died within about 3 weeks, with extensive hemorrhagic necrosis of the liver. Monkeys receiving 0.25 mg/kg twice weekly or 62 mg/kg once weekly survived up to 2 yr; all animals, even those at the lowest dose level, had liver injury. Dietary protein levels did not influence the response, perhaps because of the low toxin dosage.

Svoboda et al. *(125)*, investigating the cellular damage of aflatoxin B_1 to rat and monkey livers, demonstrated that the damage in rat liver was usually periportal; the monkey liver showed a necrosis similar to that observed in human liver during acute viral hepatitis. Svoboda's studies were paralleled by Kelly et al. *(126)*, who investigated liver tumors induced in monkeys by *N*-nitrosodiethylamine; prior to tumor formation, the pathologic changes resembled those observed in the livers of animals suffering from hepatitis.

Hazard to Man

Based on studies of chemical carcinogenesis in recent years, Boyland *(127)* suggests that chemical compounds cause at least 90% of cancer in man, and that naturally occurring substances induce 80% or more of the cancers found in Western peoples. Various surveys of worldwide mortality from cancer also indicate that environmental factors exert considerable influence; mortality rates are not uniform,

depending on the region and the type of population. The same applies to liver disease. Oettle *(128)* reviewed the earlier concepts concerning the incidences of liver tumors in different ethnic groups; malnutrition, liver parasites, viral hepatitis, and chronic alcoholism are possible causitive factors, along with senecio alkaloids and several other environmental toxins.

Shortly after the aflatoxins were discovered, the question arose whether mycotoxins—and particularly aflatoxin—might be associated with high incidences of liver disease, including liver carcinoma. Typical for the investigative approach to the problem was the study of peanuts. Contamination of peanuts was found in almost all regions of the world *(1, 129)* including the United States *(56)*. Being a major source of protein in many African communities, peanuts were examined for contamination, especially in view of the very high incidence of liver cancer among several African populations. The Tropical Products Institute in England tested peanut samples from Africa, Latin America, and Asia; less than 50% contained significant amounts of aflatoxin B_1. On the other hand, peanut meal and peanut cake were contaminated to a higher extent, 42% containing over 0.25 ppm aflatoxin B_1 *(130)*. Purchased on the open market in Uganda, about 15% of the peanut samples were contaminated with more than 1 ppm aflatoxin B_1 *(131)*. In South Africa, the yearly peanut crops often contained high levels of aflatoxins *(132)* (Table 7).

Aflatoxins were isolated from virtually every staple food consumed by man *(77, 133, 134)*, and comprehensive studies confirmed the presence of aflatoxins in virtually every area of the world, as discussed in the section on epidemiology of *Mycotoxins in Human Health (1)*.

The toxins are most often found in areas like southeast Asia or parts of Africa where climatic conditions are especially conducive to mold growth and where the methods for harvesting and storage are rather primitive. Now it appears certain that the regional incidences of hepatomas are related to tropical climate, malnutrition, and the consumption of foods contaminated with aflatoxin and other substances *(135)*.

Several investigators *(89)* presented evidence that the correlation was not totally valid in every instance and pointed out that the evidence suggesting an etiological relationship between mold metabolites and cancer was, at best, presumptive. The most useful studies, therefore, are those dealing with specific populations where a possible relationship existed between aflatoxin consumption and liver disease.

For example, Campbell and Salamat *(136)* reported high consumption of aflatoxins from peanuts and other foods in the Philippines (Table 8). Studying many members of a population that manifested an apparent correlation between a high incidence of liver disease and consumption of aflatoxin-contaminated peanut butter, Campbell et al. *(137)* found that about 15 μg of aflatoxin B_1 had to be ingested in order for M_1 to be detected in a 24-hr urine sample. In these studies, neither aflatoxin nor its metabolites were detected in human milk.

Table 7. Incidence of Aspergillus flavus and of aflatoxin above 0.05 ppm in foodstuffs of Swaziland. [a]

Foodstuffs	No. of samples	Frequency of *A. flavus*, %	Frequency of samples with aflatoxin, %
Maize	256	53.5	1.6
Groundnuts	180	49.4	11.1
Groundnut meal	48	50.0	8.3
Sorghum	39	33.3	7.7

[a] Data of Martin et al. *(132)*.

Table 8. Aflatoxin analyses of Philippine foods (1967-1969). [a]

Food	No. of samples	No. greater than 30 μg/kg	Median (and highest) value of samples, μg/kg [b]
Peanuts, whole	71	5	17 (100)
Peanut butter, Philippine (1967–68)	29	29	155 (8600)
Peanut butter, imported from U.S.A	3	0	—
Other peanut products	32	11	37 (220)
Nuts and seeds	23	1	38 (64)
Tubers	59	6	68 (440)
Beans	29	2	45 (86)
Soybean products	24	0	16 (16)
Rice and rice products	72	1	16 (33)
Maize products (1967-68)	14	1	12 (39)
Maize products (1969)	27	14	47 (400)
Cocoa	11	0	19 (29)
Livestock feeds	11	8	74 (103)
Fish products	27	0	—
Cocoanut products	7	0	21 (26)
Cooking oil	16	0	—
Mango	12	0	—

[a] Data of Campbell and Salamat *(136)*.
[b] Values above 10 μg/kg.

Robinson *(138)* reviewed the clinical history of infantile cirrhosis in India and discussed several possible etiologic factors including mycotoxins, microbial infections, viral hepatitis, and ingestion of various toxins. He examined 43 samples of milk from mothers of cirrhotic children and discovered that a significant number had fluorescent spots and R_f values that indicated contamination and aflatoxin B_1. In addition, 18 of 50 urine samples from cirrhotic children were positive for B_1.

Recent publications by Shank et al. *(129, 139–145)* provide compelling evidence that aflatoxins play a role in both chronic and acute liver disease in populations in southeast Asia. Particularly convincing is the evidence in children dying of an acute encephalopathy and fatty degeneration of the liver and kidney; significant amounts of aflatoxin are present in the tissues of these patients at autopsy *(145)*.

Influence of Nutrition on Response to Aflatoxin

The influence of nutrition is particularly interesting, because malnutrition, aflatoxins, and a high incidence of liver diseases, including liver cell cancer, coexist in many populations.

The influence of dietary protein was investigated by many laboratories, but the results were not consistent. Madhavan et al. *(121)* speculated that undernourishment and protein deficiency, causing kwashiorkor in children, might be a basis for the induction of infantile cirrhosis when aflatoxins were superimposed. To test the effect of dietary protein, they designed experiments in monkeys and observed that reduced protein intake significantly increased the susceptibility of monkeys to aflatoxin. When dose levels of 100 μg/day were administered to both protein-deficient and control animals, all members of the protein-deficient group died, whereas those receiving adequate protein survived. This implied that, conversely, a high-protein diet in the monkeys would preclude severe injury from aflatoxin. Although the control animals on a normal protein diet showed no effect after one month's exposure to 100 or 250 μg aflatoxin per day, the monkeys showed periportal fatty degeneration of the liver after a 6-month exposure. Rhesus monkeys kept on either high or low protein diets and exposed to 500 μg aflatoxin daily developed fatty livers and biliary fibrosis in 16–30 days. Monkeys on low-protein diets which received only 100 μg of toxin daily showed similar liver changes, again indicating that the protein level of the diet might significantly influence the response to aflatoxin.

Similarly, Newberne et al. *(146, 147)*, working with rats given a total dose of 375 μg aflatoxin B_1 over 3 weeks, observed that animals given diets containing 9% protein suffered a higher incidence of liver tumors in a shorter period of time than did rats receiving diets containing 22% protein. Madhavan and Gopalan *(148)* reported that rats fed a low-protein diet (5%) were more sensitive to the toxic effects of aflatoxin but less sensitive to the hepatocarcinogenic effects than those fed a 20% protein diet.

Foy et al. *(149)* discussed the similarity between carcinoma in Africans and the aflatoxin-induced hepatic cirrhosis in baboons suffering from pyridoxine deficiency; in addition to bile duct hyperplasia, the pyridoxine-deficient baboons also revealed disturbances in the liver content of lipid, glycogen, RNA, and DNA. The changes resembled those induced by aflatoxin. Thus, these investigators suggested that the very high incidence of liver cirrhosis and liver carcinoma in certain African populations might result from diets that were deficient in pyridoxine and contained aflatoxin from contaminated food; the local beer was often contaminated with aflatoxin.

Aflatoxin together with pyridoxine deficiency appear to induce liver damage. It seems probable that the reduction in pyridoxine impairs the capacity of liver cells to carry out many of the reactions essential to normal homeostasis, particularly transamination reactions; these matabolic changes may sensitize the liver to the toxic effects of aflatoxin.

Newberne et al. *(146)* determined the influence of cirrhosis induced by lipotrope deficiency on the sensitivity of rats to low levels of aflatoxin B_1. The concurrent application of dietary and toxic injuries increased tumor incidence. Animals returned to a normal diet after cirrhosis induction and then exposed to aflatoxin at a level of 1 ppm developed tumors at an even higher incidence. This seemed to imply that, once the initial biochemical lesion was induced by the toxin, a return to normal diet actually speeded up the carcinogenic process. Other forms of injury in this study, such as repeated biopsy, did not affect the induction of liver cell carcinoma.

The populations in several areas with a high incidence of liver carcinoma also suffer from marginal lipotrope — methionine and vitamin B_{12} — deficiencies. Laboratory studies indicate that marginal deficiencies of lipotropes may significantly influence the response to aflatoxin.

Rogers and Newberne *(150, 151)* showed that rats with marginal lipotrope deficiency were protected against the acute effects of a single dose of aflatoxin B_1 (Table 9); they speculated that this protection resulted from the low resting level of drug-metabolizing enzymes observed in the animals. However, if this same total amount of aflatoxin B_1 was administered in several daily doses, the rats were considerably more sensitive (Table 10). Furthermore, deficient animals treated with carcinogenic doses of aflatoxin B_1 developed liver tumors in a higher incidence and in a much

Table 9. Effects of diet on acute toxicity of a single dose of aflatoxin B_1.[a]

Animal	Dosage, mg/kg [b]	Route of administration	Diet	2-week mortality	
				No. of rats	%
Sprague-Dawley rats	7	Intragastric	Control	3/5	60
			Marginal lipotrope	0/5	0
	9	Intragastric	Control	4/5	80
			Marginal lipotrope	0/10	0
	7	Intraperitoneal	Control	5/5	100
			Marginal lipotrope	0/5	
Fischer rats	7	Intragastric	Control	10/10	100
			Marginal lipotrope	0/10	

[a] Data of Newberne, and Rogers *(152)*.
[b] Aflatoxin B_1 dissolved in 0.1 ml DMSO.

Table 10. Effect of lipotropes on parenchymal hyperplasia and tumor incidence induced by aflatoxin B_1.[a]

Dietary treatment	Appearance of hyperplastic cell clusters	Tumor incidence at various times after exposure to aflatoxin, %		
		6 months	9 months	12 months
Control	6 months	0	30	71
Severe deficiency	none	0	50	60
Marginal deficiency	3 weeks	40	95	95

[a] Data of Newberne and Rogers *(152)*.
[b] Rats were given 15 daily doses, 25 μg, each, of aflatoxin B_1.

shorter period of time than did controls. Possible mechanisms for these interactions triggered an ongoing intensive investigation.

Newberne and Rogers *(153)* reported that low dietary vitamin A apparently decreased the induction of liver tumors. Despite this protection from the carcinogenic effects of aflatoxin, however, the vitamin A-deficient rats had a much higher mortality rate and their growth rates differed somewhat from animals receiving adequate vitamin A. Furthermore, colon carcinomas were observed in a significant number of deficient rats. Clearly, the influence of vitamin A on aflatoxin-induced tumors posed an important problem requiring further investigation.

Influence of Factors Other Than Diet

Goodall and Butler *(154)* studied the effects of hypophysectomy on rats fed a diet containing 4 ppm aflatoxin B_1. All of the control animals developed liver tumors in 49 weeks, whereas none of the hypophysectomized animals developed tumors in the same period of time. Hypophysectomized rats, however, grew little if any over the period of the experiment, whereas the other animals grew normally. This discrepancy rendered the interpretation of results exceedingly difficult. Similar observations, however, were made under analogous conditions in experiments with aminofluorenes *(155)*.

Newberne and Williams *(156)* reported that rats fed diets containing 0.2 ppm aflatoxin B_1 and 4 ppm diethylstilbestrol developed fewer liver tumors than those that were treated with aflatoxin alone. The study included paired-feeding and feeding-to-weight, indicating that decreased tumor incidence associated with diethylstilbestrol was not the result of decreased food intake. The specific mechanisms responsible for protection remained obscure. Further experiments *(157)* showed that methionine fed at 0.2% of the diet acted synergistically with aflatoxin B_1 at 0.4 ppm to induce liver cancer in rats. However, aflatoxin B_1 alone at dietary levels of 0.4 of 1.5 ppm was more effective in inducing liver tumors than in combination with urethane at 0.1–0.6% of the diet. Studies in which rats were exposed to rubratoxin B and to aflatoxin B_1 indicated a sensitization to rubratoxin, but the evidence for potentiation of carcinogenesis was equivocal *(49)*.

Reddy and Svoboda *(158)*, studying the effect of lasiocarpine given to rats with and without aflatoxin, found that the pyrrolizidine alkaloid (lasiocarpine) was a hepatocarcinogen in itself; moreover, it modified the morphologic pattern of livers in which tumors developed. It also resulted in a postnecrotic cirrhosis which is not characteristic of aflatoxin alone. Newberne and Rogers *(159)* found that another pyrrolizidine alkaloid, monocrotaline, also was carcinogenic for the rat and that it also modified the histologic structure of livers in which tumors developed as a result of exposure to both aflatoxin and monocrotaline. As in earlier studies in which lasiocarpine failed to inhibit the induction of liver tumors by acetylaminofluorene *(160)*, monocrotaline failed to prevent the induction of liver cell tumors by aflatoxin, despite its potent antimitotic effect *(161)*. Monocrotaline was more potent as a syncarcinogen when the diet was low in lipotropes.

McLean and Marshall *(162)* investigated the influence of phenobarbitone on the induction of liver cancer by aflatoxin B_1. The aflatoxin was fed to rats at 5 ppm in the diet for 9 weeks; during this period the experimental group received continuously phenobarbitone in water. The rats receiving phenobarbitone and aflatoxin had a lower incidence of liver tumors and a longer time lag in tumor development in comparison to rats fed aflatoxin alone. McLean and Marshall suggested that phenobarbitone induced liver microsomal enzymes that metabolized aflatoxin to a noncarcinogenic form or somehow accelerated its excretion.

Control of the Hazard

Probably the most realistic way to preclude public health hazards is to prevent aflatoxin formation on foodstuffs in the first place. In order to achieve this goal, the farmer must cooperate at harvest time, the grain must be removed rapidly from the ground, and the grain must be stored and processed under conditions which are not conducive to mold growth. The University of Wisconsin has a useful report on the prevention of toxin-producing molds in farm commodities *(163)*.

When aflatoxins are present, however, it is mandatory to devise means for the inactivation or detoxification of contaminated materials supplying nutrition to man and animals. Detoxification generally relies on biological, chemical, or physical removal of the compounds, or on their chemical or physical inactivation *(4, 164)*. The details of these procedures are described by Detroy et al. *(62)* and by Dollear *(165)*.

In the United States, the Food and Drug Administration continuously scrutinizes many staple foods, particularly oil seeds and cereal grains, for the presence of aflatoxins; ceaseless efforts are being made to elucidate the biological action of mold toxins. The U.S. Department of Agriculture plays a major role in making information about mycotoxins available. The former widespread contamination of food products—peanuts, cottonseed meal, soybeans, rice, wheat, and the majority of cereal grains—is now under control through improved

harvesting methods and through surveillance by the USDA and FDA, greatly reducing the potential health hazard in the United States. The not so closely monitored animal feeds, however, still pose a problem.

In those areas of the world where climate, harvesting, and storage permit or enhance mold growth, mycotoxins, particularly those produced by *A. flavus*, still present a public health problem. It is clear that newer and better methods of harvesting and storing decrease the contamination, and that the introduction of these methods is essential for solving the problem around the world.

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