CHAPTER 6

Fungal Toxins Occurring in Foods

I. Mycotoxins

Fungi produce a multitude of substances of wide-ranging chemical structure and biological activity. Certain fungal metabolites are highly desired components in some foods such as cheese, while other metabolites are important antibiotics—penicillin, for instance. However, some fungi can produce substances that are potent acute toxins or carcinogens to both animals and humans. These toxic agents are generally called mycotoxins, a term usually reserved for toxins produced by filamentous fungi. The diseases these fungi cause are mycotoxicoses; their impact on domestic animals in terms of decreased growth rate, abnormal reproduction, disease, and early death has been known for centuries. The impact of these fungi in human illness has also been recognized for centuries, but their role as possible human carcinogens has been the subject of intensive study only since the early 1960s.

A. Ergotism

An association between the consumption of certain grains and human diseases was made early in history. Sacred writings of India (300—400 B.C.) refer to noxious grasses that caused pregnant women to abort or die in childbirth. Julius Caesar, in the first century B.C., is reported to have identified spoiled grain as the cause of certain epidemics that continued to occur regularly up to the Middle Ages in Europe.

By the middle of the 17th century, a cause and effect relationship was established between the consumption of grain contaminated with the fungus *Claviceps purpurea* (ergot) and disease. Under conditions of adequate moisture and warmth, the fungus invades individual grain kernels and form a sclerotium. The sclerotium, a lightly curved, black to purple body up to 6 cm long, is the resting stage of *Claviceps* and can remain viable under dry conditions and germinate when moistened. Today it is established that the growth of as many as 50 species of *Claviceps* on various food and feed crops in the grass family is associated with ergotism, the disease syndrome produced by ergot.

Gangrenous-type ergot poisoning may include severe pain, a burnt appearance of the affected limbs, and an inflammation of the extremities, which then turn black and, in severe cases, separate from the body. Because of the burnt appearance symptom, ergotism was called “holy fire” or “St. Anthony’s Fire” in the Middle Ages from the belief that St. Anthony offered relief to those afflicted with the disease. A second type of ergotism that occurs under certain conditions is known as convulsive ergotism; it is characterized by neurological disorders such as numbness, blindness, paralysis, and convulsions.

The physiological basis of the gangrenous type of ergotism is the constriction of blood vessels. This activity has been exploited for centuries by the use of low doses of ergot to stop hemorrhage at childbirth. The blood vessel constriction results from a general stimulation of smooth muscles that is due to the direct effect on the muscle by ergot without the intervention of chemical or nervous mediators.

Ergot has also been used to induce uterine contraction during pregnancy. However, since uterine muscle is generally no more sensitive than other smooth muscle to the constrictive effects of ergot, a dangerously high dose must be used to induce uterine contraction in early pregnancy. However, uterine muscle becomes sensitive in the advanced stages of pregnancy and responds to much lower doses than the other smooth muscles during this period.
Components of ergot are also used today as medications for certain complications during pregnancy.

The physiological basis for the selective occurrence of convulsive ergotism is poorly understood. Several hypotheses have been proposed, however, including the involvement of nutritional status of the afflicted individual, biosynthetic variability of the ergot fungus, genetic differences in susceptibility to ergot, and the possibility that another fungus may be parasitizing the ergot and introducing neurologically active substances. Resolution of these various possibilities requires further extensive research.

The principal pharmacologically active substances of ergot are a series of alkaloid derivatives that have lysergic acid as a part of their basic structure (Figure 6.1). Most important of the ergot alkaloids are ergotamine, ergonovine, and ergotoxin. Ergotamine was first isolated by Stowell in 1918 and is the first pure ergot alkaloid to find widespread medical application. Ergotamine tartrate is used almost exclusively in the treatment of migraine and other vascular headaches. Its mode of action is believed to involve vasoconstriction. Although the substance is very

\[ \text{Lysergic acid: } R = \text{OH} \]

\[ \text{Ergonovine: } R = \text{N–CH} \]

\[ \text{Ergotamine: } R = \text{N–CH}_2\text{OH} \]

Figure 6.1 Structures of lysergic acid and related compounds.

effective against migraines, it is not suitable for long-term prophylactic use because of serious adverse effects such as severe vasoconstriction, resulting in dry gangrene of the extremities. Ergotamine is thought to be one of the substances primarily responsible for the characteristic gangrenous effect observed in ergot poisoning.

Ergonovine was first isolated in 1935 and found to be a potent inducer of uterine constriction. Ergonovine also causes significant vasoconstriction but does not exhibit the adrenergic blocking action of ergotamine. Ergonovine and a derivative, methyl ergonovine, are used in obstetrics in the third stage of labor, principally to decrease postpartum bleeding.

Ergotoxin is a crystalline mixture of ergocrinine, ergokryptine, and ergocornine, all of which are similar in
structure to ergotamine. The crystalline form of ergotoxin was first isolated from ergot in 1906. The ergotoxin group, like ergotamine, affects smooth muscle action and can block norepinephrine and epinephrine. A hydrogenated preparation of ergotoxin is useful in the treatment of peripheral and cerebral vascular disorders and essential hypertension. Synthetic amide derivatives of lysergic acid, including LDS, are highly potent hallucinogens in humans and as such are under intensive, continuing study.

**B. Alimentary Toxic Aleukia**

Alimentary toxic aleukia (ATA), or septic angina, is another mycotoxicosis that has caused great human suffering. ATA has been reported from time to time, primarily in Russia since the 19th century. Outbreaks were recorded in 1913, 1932, and toward the end of World War II. Russian accounts of symptoms of the disease include fever; hemorrhagic rash; bleeding in the nose, throat, and gums; necrotic angina; extreme leukopenia; agranulocytosis; sepsis; and modification of the bone marrow. Outbreaks of the disease were generally sudden, and mortality rates were often in excess of 50% of those afflicted. Russian scientists have identified four stages of the disease. The symptoms of Stage 1 generally appear shortly after consumption of the toxic food; they include burning sensations in the mouth, throat, esophagus, and stomach. This may be followed by vomiting, diarrhea, and abdominal pain due to inflammation of the gastric and intestinal mucosae. Patients at this stage also experience headaches, dizziness, fatigue, tachycardia, salivation, and fever; additionally, the blood leukocyte count is reduced. The symptoms may last from 3 to 9 days and then subside, signalling the onset of Stage 2. With the reduction in the intensity of the symptoms from the first stage, the patient may feel well and be capable of normal activity. However, at this stage, destruction of the blood-cell forming system progresses and there is continued reduction in lymphocytes accompanied by anemia. The body’s resistance to bacterial infection is reduced; general weakness, headache, and reduced blood pressure are apparent. The duration of this second stage may be several weeks to months. If consumption of toxic food is stopped at this point and the patient is hospitalized, chances of recovery are very good. However, continued consumption of toxic food will lead to the onset of Stage 3. Stage 3 is signalled by the appearance of hemorrhages on the skin and on the mucous membranes of the mouth and tongue as well as in the intestine and stomach. As the toxicosis progresses, the necrotic lesions proliferate along with an increased tendency for bacterial infection. Esophageal lesions often occur and the involvement of the epiglottis may cause laryngeal edema leading in many cases to strangulation. Destruction of the hematopoietic system continues at this stage. If the patient survives Stage 3 by, for example, blood transfusions and administration of antibiotics, Stage 4, or the period of convalescence, is begun. Stage 4 often requires several months for the complete recovery of the hematopoietic system.

After rejecting several theories concerning the cause of the disease, such as vitamin deficiencies or infections, investigators eventually discovered that the disease was caused by consumption of grain left in the fields over the winter. Examination of the fungal flora of wintered cereals implicated in outbreaks of ATA revealed a rich array of fungal species. Toxicities of purified fungi, determined by application of fungal extracts to the skin of rabbits, were then associated with toxicity of moldy cereals to people. The rabbit skin assay was used for identification of toxic fungal strains and for subsequent efforts to identify active substances.

Fungi of the genus *Fusarium* were shown to produce the highest number of toxic isolates in the rabbit skin assay. The species most often associated with ATA outbreaks were *Fusarium poae* and *Fusarium sporotrichoides* (also called *Fusarium tricinctum*), which are fairly common species of fungi that have the peculiar capability of producing toxic substances in increased amounts when grown under conditions that include a period of growth at temperatures near 0°C.

In early studies, attempts to define the systematic sequence of ATA toxicosis were complicated by the variability of the disease. This variability was found to be due to the quantity and toxicity of material ingested, as well as other factors such as the age and nutritional status of the affected individual.

Early efforts by Russian scientists to isolate toxic substances responsible for ATA resulted in the identification of two steroidal compounds, one called sporofusarin from *Fusarium sporotrichoides* and another called poaefusarin from *Fusarium poae* (Figure 6.2). Efforts in the United States and elsewhere to isolate these substances from toxic *Fusarium* species were unsuccessful. Instead, substances of the trichothecin class were isolated (Figure 6.3).
More recent efforts to confirm the toxicity of poaefusarin have shown that the sample contained enough trichothecins to account for the toxicity of the extract. In addition, examination of trichothecins produced by \textit{F. poae} and \textit{F. sporotrichoides} implicated in outbreaks of ATA established the production of toxic trichothecins by toxic isolates of these species. T-2 toxin (Figure 6.4) isolated from \textit{F. sporotrichoides} and administered orally in gelatin capsules to cats produced all of the toxic symptoms characteristic of ATA. Removal of T-2 toxin from the \textit{Fusarium} extract results in the loss of toxicity of the extract.

Although these studies clearly implicate T-2 toxin as a possible causative factor in the hemorrhagic lesions of ATA, results of other studies have suggested a more complicated etiology. For example, T-2 toxin administered orally to pigs and cats at a dose of 0.2 mg/kg body weight over a period of 78 days failed to induce clinical hemorrhagic syndromes. Chronic administration of T-2 toxin (20 ppm) to young mice showed that this species was susceptible both to the irritant and hematopoietic suppressive effects of dietary T-2 toxin, but that the suppression of hematopoiesis was transient and did not lead to hematopoietic failure.

The variability in response of various species to T-2 toxin may be due to several factors. These include

1. some species may be resistant to the suppressive effects of dietary T-2 toxin;
2. other toxins may be more important than T-2 toxin in the naturally occurring mycotoxicoses; and
3. dietary and other factors (including different mycotoxins) may increase the toxicity of T-2 toxin.
Thus, although tricothecenes such as T-2 toxin probably play a role in the etiology of ATA, the disease cannot be ascribed to a single substance and the complex interaction of trichothecenes with host variables must be unraveled before the etiology of ATA can be understood.

C. Aflatoxins

Moldy food has long been associated with various diseases in animals. These diseases were generally thought to be problems of livestock yield for farmers and the possible implications for human health were generally not considered. Various liver diseases, dubbed collectively “hepatitis—X” disease, were diagnosed by veterinarians in swine, cattle, and, in some cases, dogs. Improved methods of feed handling, production, and storage considerably reduced the occurrence of these types of diseases. It was not until 1960 that the human health implications of farm animal diseases began to be clearly recognized. At that time, over 100,000 young turkeys in England died of a disease that was dubbed “Turkey—X” disease. The disease was characterized by extensive necrosis of the liver in turkey poultis. At the same time, attention was drawn to an increasing incidence of hepatic tumors in trout raised in hatcheries in the United States. It was later shown that both the groundnuts (peanuts) used as a feed supplement for the turkeys and the cottonseed used as a feed supplement for the trout were contaminated by a series of compounds produced by a fungus known as *Aspergillus flavus*. These compounds, the aflatoxins, are not only potent acute toxins in several species, but are also some of the most potent hepatocarcinogens known.

Aflatoxins are a series of bisfuran polycyclic compounds (Figure 6.5). Based on their characteristic blue or green fluorescence under ultraviolet

![Figure 6.5 Structures of aflatoxins.](image)
light, these compounds were given the names aflatoxin B₂, G₁, and G₂, all of which are mold metabolites. Hydroxylated derivatives of aflatoxin B₂ and G₂ have also been isolated from mold and given the names aflatoxin B₂a and G₂a respectively. The aflatoxins are lipid soluble and are not destroyed by most normal cooking conditions. They are also generally unstable when exposed to ultraviolet radiation.

1. **Occurrence of Aspergillus flavus**

*Aspergillus flavus* is a common constituent of the microflora in air and soil throughout the world. It causes deterioration of stored wheat, corn, rice, barley, bran, flour, and soybeans. In general, it does not invade the seeds of living wheat or intact peanuts. Growth occurs primarily when products are stored under conditions of relatively low moisture that eliminate the growth of competing species such as *Penicillium* and *Eusarzum*.

With the development of sensitive assays for aflatoxins, many feed and food crops were analyzed for their presence. Assays conducted in the early 1960s in the United States showed that aflatoxins were present in the majority of peanuts and peanut meals produced in the United States and elsewhere. These assays also showed that roughly half of the peanut butter samples collected were contaminated with aflatoxins. Analyses of food samples collected from around the world, particularly from Africa and Asia, showed that aflatoxins could be detected in barley, cassava, corn, cottonseed, peas, millet, cowpeas, rice, sesame, sorghum, soybean, sweet potatoes, and wheat. Even dried spaghetti can contain aflatoxins. It is likely that aflatoxins are present in food and feed that are stored under conditions with enough moisture to allow the growth of *A. flavus* but not enough moisture to prevent the growth of other organisms.

2. **Metabolism**

Aflatoxin B₁ metabolism (Figure 6.6) has been studied in many species and under many different conditions. Aflatoxin B₁ is converted to at least seven metabolites, including a proposed unstable metabolite, the 8,9-epoxide, which is the so-called ultimate carcinogenic form (vide infra). Aflatoxin M₁ occurs in milk of cows fed on aflatoxin B₁-containing feeds.

![Figure 6.6 Metabolic pathways of aflatoxins.](image-url)
This metabolite is found in the liver, kidneys, and urine of sheep and in the livers of rats treated with aflatoxin B₁. The total conversion of aflatoxin B₁ to M₁ in cow’s milk is estimated to be about 1%. In comparing carcinogenic activity in rats, aflatoxin M₁ is less than one-tenth as active as aflatoxin B₁. However, the acute toxicities of these substances are roughly similar. Aflatoxin M₁ generally comprises less than 2% of the total aflatoxin metabolites in the body.

Aflatoxicol, a reduction product of aflatoxin B₁, has roughly one-twentieth the acute toxicity of aflatoxin B₁ in the duckling bioassay but it has about one-fifth the mutagenic activity of aflatoxin B₁ in the Ames assay and roughly half the carcinogenic activity in trout. In vivo, it is readily oxidized to aflatoxin B₁ and may also serve as a reservoir for aflatoxin B₁. However, since the rate of aflatoxicol production in various test organisms is not consistently related to susceptibility to aflatoxin poisoning, its role in aflatoxicosis is unclear.

Two other major metabolites of aflatoxin B₁ are aflatoxins P₁ and Q₁. These substances have much lower acute toxicity than does aflatoxin B₁. Aflatoxin Q₁ is nontumorgenic in trout.

Evidence for the production of aflatoxin B₁-8,9-epoxide so far has been indirect but nevertheless substantial. Results of metabolic studies have shown that under certain circumstances an aflatoxin B₁-8,9-dihydro-8,9-diol can be isolated. In addition, an aflatoxin B₁ derivative has been isolated which is a likely product of binding of aflatoxin B₁-8,9-epoxide to guanine residues of nucleic acids (Figure 6.7).

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\text{Figure 6.7 Formation of aflatoxin epoxide and guanine adduct.}
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3. Toxicities

Ducklings are the most sensitive species to the acute toxic effects of aflatoxins. The toxic potencies of aflatoxin B₁ in this assay are indicated in Table 6.1. Trout is also a sensitive species to the acute effects of aflatoxins, whereas rats—and in particular, female rats—are relatively insensitive to the acute effects. Prominent acute effects in rats include hepatic lesions with edema, biliary proliferation, and parenchymal cell necrosis. In rhesus monkeys, aflatoxin treatment commonly results in fatty infiltration and biliary proliferation with portal fibrosis. There is considerable species variation in acute and chronic effects of aflatoxins. With regard to the chronic effects of aflatoxins, a high percentage of tumor induction was seen in male Fisher rats fed 2 ppm of aflatoxin B₁, while no tumor induction was seen in male albino mice fed aflatoxin B₁ at the same level. Sensitive species such as rats and trout show significant tumorigenesis when aflatoxin B₁ is fed at levels less than 100 ppb.

With the advent of sensitive analytical techniques for measuring aflatoxin levels in various crops and with a realization of the highly hazardous nature of these substances, the FDA has set limits on aflatoxin levels in food and feeds. Currently, the action limit for aflatoxins in milk is 0.5 ppb and in most foods it is 20 ppb. The maximum allowable limit for aflatoxins in animal feed is set at 100 ppb. Under these guidelines, millions of dollars worth of contaminated food and feed products have been seized by the FDA.

4. Detoxification

Many methods have been used in an effort to detoxify contaminated feeds. Physical separation of obviously contaminated materials has proven successful in controlling aflatoxin contamination in peanuts. Aspergillus flavus and several other fungi emit a bright yellow-green fluorescence under ultraviolet light. This telltale signal of fungal contamination has been useful in the physical separation of contaminated peanuts and corn as well as a few other crop samples.

Heat treatment of contaminated crops has also been used to detoxify food or feed material. Generally, under dry conditions the aflatoxins are quite heat stable. However, normal roasting conditions reduce the aflatoxin B₁ content in peanuts by 80% after half an hour. Heating under conditions similar to the moist conditions used for autoclaving are much more effective in reducing aflatoxin content than dry heating. This method has met with limited use, however. Several methods of solvent extraction have been employed with limited success. These methods are quite costly and time consuming and toxic components are not totally removed. In addition, essential nutrients may be extracted from the food or feed.

Several chemicals (such as hydrogen peroxide, ozone, and chlorine) have been used to destroy aflatoxins. These substances react readily with aflatoxins in foods as well as with many desired substances, including vitamins. The efficacy of these reagents in producing nontoxic yet nutritious material has not been established.

A more useful method of chemical detoxification of contaminated feed is treatment with ammonia. The use of ammonia to detoxify corn meal and cottonseed meal increases the nutritional value of the feed. The detoxified feed supports the growth of trout, cows, and other animals without apparent ill effects. An ammoniation process developed in Arizona involves placing a mixture of aqueous ammonia and cottonseed in large plastic bags commonly used for silage. The bags are sealed and allowed to stand in the sun for several weeks. The process has
been shown to be effective in reducing the levels of aflatoxin in highly contaminated cottonseed (800 ppb) to less than the 100 ppb action levels set by the FDA.

5. General Considerations

Studies of the importance of aflatoxins in the etiology of human cancer have concentrated primarily on areas of Southeast Asia, China, and Africa where aflatoxin levels in the food are relatively high. Continuing analyses of the combined epidemiological data from several such studies indicate that high-level intake of aflatoxin in combination with such other diseases as hepatitis is associated with an increased rate of liver cancer. Whether aflatoxins by themselves are human hepatocarcinogens remains the subject of scientific controversy. Despite these uncertainties about the role of aflatoxins in human cancer, efforts to minimize human exposures continue. There are well-established methods of harvesting, drying, and storing crops that are effective in the control of fungal contamination and aflatoxin production. Efforts are ongoing to implement these techniques in areas of high aflatoxin contamination in the hope of reducing the incidence of liver cancer.

II. Other Mycotoxins

Following the dramatic discovery of the aflatoxins, many investigators initiated studies on other fungi that occur on food and feed. Many toxins have been isolated from other fungi but their effects are much less dramatic than those of the aflatoxins and they have not been as thoroughly studied. A general screening program with ducklings as the test organism showed that most of the strains of *Aspergillus ochraceous* were nondgenic. *Aspergillus ochraceus*, a mold similar to *A. flavus*, occurs widely in nature and is found in the soil and on decaying vegetation. Toxic substances isolated from *A. ochraceus* include ochratoxins A and B (Figure 6.8). *Aspergillus ochraceus* is a predominant fungus on red and black peppers and is found in stored cottonseed, citrus fruit, peanuts, and tobacco. The fungus has also been used in Japan to produce fermented fish, which is known as katsuo bushi. In albino rats, ochratoxin A has an LD<sub>50</sub> of 20 mg/kg. It produces liver degeneration but it is apparently not carcinogenic. Ochratoxin B is much less toxic. The ochratoxins are hydrolyzed in the liver and secreted in the bile.

Rubratoxins (Figure 6.9) are acute toxins produced by *Penicillium rubrum* with apparently no carcinogenic effects. The LD<sub>50</sub> of rubratoxin A is 6.6 mg/kg by intraperitoneal injection in rats. Administration of these toxins to animals causes extensive liver and kidney damage.

Considerable work has been done in Japan on the potential hazard of moldy or yellowed rice. Many fungi and many of their toxic metabolites have been isolated. Yellowed rice toxins are mentioned here because two implicated fungi, *Penicillium islandicum* and *Penicillium rugulosum*, are the first non-*Aspergillus* species identified which produce tumorigenic substances.

![Figure 6.8 Structures of ochratoxins.](image)
Administration of approximately 200 g/day of rice infected with *Penicillium islandicum* for 1 week causes the death of laboratory animals from liver necrosis. In another study, a high percentage of animals developed liver tumors following 2 years of consuming a diet that contained a 0.05 g/day of moldy rice. Few malignant tumors were observed, however. Three of the active components in the yellowed rice are rugulosin, luteoskyrin, and islandicin (Figure 6.10). Liver necrosis is the primary cause of death in mice following treatment with rugulosin (LD₅₀ - 83 mg/kg) and luteoskyrine (LD₅₀ - 7 mg/kg). Prolonged feeding of luteoskyrine to mice at a relatively high dose (50 mg/kg) resulted in liver tumors. Islanditoxin, a highly toxic cyclic peptide produced by *P. islandicum*, causes severe liver damage, hemorrhage, and death in animals when administered in low oral doses (LD₅₀ - 7 mg/kg).

An estrogenic substance, zearalenone (Figure 6.11), is produced by the fungus *Fusarium roseum* and its sexual stage, *Gibberella zeae*, as well as by related fungi. Consumption of corn infected with these fungi causes loss of reproductive capacity and other estrogenic effects in pigs and other animals. Symptoms of zearalenone poisoning in young pigs include swelling and eversion of the vagina until in some cases the cervix is visible.
III. Mushroom Fungal Toxins

Mushrooms are a delicacy to people the world over. A few species are grown commercially in the United States and are consumed in vast quantities with considerable enjoyment. But health problems can arise from the consumption of wild mushrooms. In the United States, only about 50 of the over 800 identified species are known to produce some toxic effects in people. In most cases, an unwary collector can consume a mildly toxic species of mushroom and most likely suffer only simple gastrointestinal upset that will soon pass. For various potentially toxic mushrooms, special cooking processes have been developed to render them edible. Only a few species are considered highly toxic or lethal if consumed. One genus in particular (Amanita) contains some of the most notorious as well as the best tasting mushroom species.

*Amanita muscaria* is a classic example of a psychoactive and toxic mushroom. This fleshy fungus grows throughout temperate areas of the world. It is not sought after as a food; instead, it has been used for many centuries as a hallucinogen. The pleasant effects of this mushroom and the reported slow degradation of the active principles combine to make *A. muscaria* a prized component of tribal and religious rituals in many parts of the world. Use of *A. muscaria* as a narcotic or intoxicant is well documented. Substances primarily responsible for the narcotic-intoxicant effect are a series of isoxazoles, such as muscimol (Figure 6.12), that comprise approximately 0.20% of...
the dry weight of *A. muscaria*. The neurological symptoms of individuals who have consumed *A. muscaria* vary, but they generally begin to occur 30—90 mm following ingestion. A state resembling alcoholic intoxication is generally produced. Confusion, restlessness, visual disturbance, muscle spasms, and delirium may follow. Patients are reported to pass into a deep sleep following the excited period, and upon waking, they may have little or no memory of the experience. Ingestion of 15 mg of pure muscimol by a single individual

![Figure 6.12 Structures of muscimol and ibotenic acid.](image)

is reported to cause confusion, visual disturbance, illusions of color vision, fatigue, and sleep.

Ibotenic acid (Figure 6.12), another isoxazole present in the mushroom, is reported to produce no psychic stimulation. This compound induces lassitude and sleep that is followed by a migraine and a lesser and localized headache that lasts for weeks. The overall response in an individual is unpredictable since individual susceptibilities differ and since the levels of the isoxazole and muscarine vary in the mushroom due to environmental and genetic factors.

*Amanita muscaria* is known as fly agaric because it contains muscarine, the principal substance responsible for the fly-killing activity of *A. muscaria* and a few other mushrooms. Muscarine is a fairly simple compound (Figure 6.13) that in comparatively small doses (0.01 mg/kg) reduces blood pressure in cats. Muscarine acts like acetylcholine on the receptors of smooth muscles and glandular cells, the so-called muscarinic receptors.

The symptoms of muscarine poisoning appear within 30 mm of ingestion. Symptoms include increased salivation, lachrymation, and perspiration followed by vomiting and diarrhea. Pulse is slow and irregular and breathing is asthmatic. Death is uncommon, and patients generally respond well to atropine sulfate. Severe cases of muscarine poisoning are rare. This indicates that the levels of muscarine relative to the levels of other substances that are causing the desired narcotic effect of the mushroom are generally quite low.

*Amanita phalloides*, also known as the death cap mushroom, is perhaps the most well-known toxic mushroom. This species is said to be responsible for 90—95% of all mushroom poisoning deaths in Europe. It has also been responsible for several deaths each year in the United States, where it was fairly rare until the early 1970s, when it apparently entered the western United States in a nursery specimen from Europe. The mushrooms, or fruiting bodies, of *A. phalloides* generally appear in the late summer or fall and can stand 8” tall. The color of the relatively large cap can range from greenish-brown to yellow. *Amanita phalloides*, because of its size and close similarity to other highly edible and sought-after species of *Amanita*, is often a prize find of avid mushroom hunters.

![Figure 6.13 Structure of muscarine.](image)

*Amanita phalloides* contains several cyclic peptides that account for the toxicity of the mushroom. The substances are of two types and have been called phallodins and amanitins. The phallodins are highly toxic in tissue cultures and when injected into test animals; however, they are weakly toxic when administered orally. The amanitins have LD50’s in mice in the order of 0.1 mg/kg and are toxic when administered orally or intravenously. It has been hypothesized that the phallodins are responsible for the initial gastrointestinal phase of poisoning, whereas the more toxic but slower acting amanitins are responsible for the later phase of toxicity which occurs after 3—5 days and affects the liver and kidneys.

The principal toxic component of *Amanita phalloides* is a-amanitin (Figure 6.14) which acts specifically to inhibit an enzyme, RNA polymerase, required to synthesize messenger RNA. The cellular effects of a amanitin
include disintegration of nucleoli in liver cells, which prevents ribosome synthesis and ultimately protein synthesis. Destruction of the convoluted tubule of the kidney is also caused by a-amanitin, which diminishes kidney’s effectiveness in filtering toxic non-electrolytes from the blood.

The chemical structures of the amanitins and the phalloidins are complex indeed. The phalloidins and amanitins are cyclic peptides that contain seven and eight amino acids, respectively. There is evidence to suggest that these cyclic peptides are mere fragments of much more complex polysaccharide components. These large molecular-weight substances, which are called myriamanins, may be isolated by a mild solvent extraction of the mushrooms. Molecular masses as high as 60,000 Da have been assigned to some of these active components. The smaller cyclic peptides are obtained from these larger compounds by strong acid or alkaline treatment.

Toxic symptoms appear many hours following consumption of A. phalloides. Initial signs of toxicity are abdominal pain, diarrhea, and vomiting. If an adult has consumed at least two caps of the mushroom, death will occur from dehydration if the individual is not treated to restore proper electrolyte balance. Any individual who survives this stage of poisoning is still in jeopardy of succumbing to subsequent toxic effects. Death in most cases is due to extensive liver and kidney damage. Attempts to find antidotes for Amanita poisoning have been generally unsuccessful. However, a preparation of cytochrome c appears to be somewhat useful in treatment of toxic effects of a-amanitin. Results of experiments with mice have shown cytochrome c to be effective in improving survival of mice, even when the treatment was withheld for 8 hr following administration of the toxin. Although the mechanism of this protective effect has not been established, this treatment and others have been used successfully in cases of human poisoning. Human survival rates have increased in recent years to above 50% and are likely to increase further as our understanding of the basic enzymology of Amanita poisoning becomes more complete.