Interactions between drugs and chemicals in industrial societies.
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NEW TRENDS IN MUSHROOM POISONING

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Among the naturally growing products, mushrooms are suspected to be the most poisonous ones. Some people would not eat any mushrooms; the majority is cautious, and consume only a few species confirmed as absolutely innocuous, although the number of edible mushrooms is much greater than it is commonly assumed.

The term "poisonous" is a matter of definition. If one defines a substance as poisonous that after ingestion causes transcient irritation of the stomach abdominal pain, nausea and diarrhea then a considreable number of mushrooms are toxic. If one, however, defines a poison as a substance that, in relatively small amounts, can lead to death, only a few species exist that must be designated poisonous fungi. 95% of all lethal intoxications by ingestion are caused by specimens of the genus Amanita. Before treating the important chapter of mushroom poisoning, I would like to mention those species that in rare cases have been reported to have caused death of patients. A most recent, amply illustrated treatise on poisonous mushrooms has been compiled by Besl and Bresinzki (1).

Traditionally, fatal toxicity has been attributed to the red fly-agaric, Amanita muscaria, the mushroom of fairy tales. The reason for this is perhaps the observation that houseflies that suckled enough of the sweetened sap of the mushroom lost their mobility and eventually died. The search for the toxic ingredient muscarine, in 1869 initiated mycotoxin research. Guided by results with the isolated frog heart, the active substance muscarine was obtained from thousands of A.muscaria.

Since one carpophore contains less than 1 mg of the moderately toxic drug, it is scarcely possible that an individual could be killed by a dish of A.muscaria. Additional, physiologically active components of this fungus, muscimol and ibotenic acid, are not sufficiently toxic as to cause death by

the amounts present. Amanita muscaria, as well as A.pantherina, the panther cap, contain the amino acid ibotenic acid (γ-amino(4-hydroxy isoxazol-2)yl acetic acid), and its product of decarboxylation, muscimol as well as muscazone in moderate amounts (figure 1). These compounds were promptly recognized as the constituents responsible for the notorious, although weak, insecticidal property of these muschrooms. Toxicologists have come to believe that muscimol and to a lesser degree ibotenic acid are the elusive intoxicants responsible for the psychomotor reactions experienced after ingesting these mushroom. The hallucinogenic property of A.muscaria has been known for a long time and these mushrooms, including A.pantherina, have been reported to be eaten deliberately in some regions. A few alarming intoxications have been reported in the United States, Europe and South Africa, especially in children.

The toxicity of muscimol, as estimated from animal experiments, is not high enough as to kill an adult man even after ingestion of several kg of A.pantherina. Muscimol is presumed to be the main psychoactive agent in man. Clinical intoxication (with symptoms of gastrointestinal irritations, dizziness, irratic and sometimes maniacal behavior) lasts for several hours. The mechanisms by which ibotenic acid and muscimol alter central neural activity are concerned with the functions of the naturally occurring transmitters L-glutamic acid and its decarboxylation product γ-aminobutyric acid (GABA).

Muscarine is found in Inocybe and Clitocybe in amounts of 0.1 to 0.5% of their dry weight, i.e. more than 100-fold more than that in Amanita muscaria. The prevailing L(+) muscarine is the physiologically most active form of the four diastereomers. It is a quarternary ammonium compound, which, due to a structural similarity, mimics the effect of acetylcholine on the parasympathic nerve endings in smooth muscles and exocrine glands. The clinical symptoms of an intoxication by muscarine can be ascribed to its parasympathomimetic effect. They develop within 30 min to 2 h following ingestion of certain species of Inocybe and Clitocybe and include salivation, lacrimation, diffuse perspiration, nausea, vomiting, headache, visual disturbances like diplopia, miosis, abdominal colic, diarrhea, dyspnea, bradycardia, profound hypotension and shock. A natural antidote is atropine.

Gyromitra esculenta, the false morel "Frühlingslorchel" in German, contains derivatives of methylhydrazine. Gyromitrine, the main component, isolated in 1968 by List and Luft (2), is the formylmethyhydrazone of acetaldehyde. Analogue hydrazones of homologous aldehydes have been detected among numerous other volatile substances by Pyysalo (3). These toxic components, normally hydrolyzed and evaporated by cooking, cause liver injury

and even death by necrosis of the liver, when ingested by humans in doses of more than 0.1 g/kg, corresponding to about 1 kg fresh mushrooms. Accordingly, sale of these mushrooms is forbidden in some countries. It is most probably methylhydrazine, set free in the liver, that causes the cellular damage.

Lethal effects by renal damage after a lag period of 3-15 days must be ascribed to Cortinarius orellanus in Polland (4), to C.speciosissimus in Finland (5,6) and Scotland (7) and to C.splendens in France (8). From the former species a bipyridyl derivative, orelline, and its bis-N-oxide, orellanine, have been isolated (9). Their role in intoxication, however, has not been fully substantiated.

Paxillus involutus can cause death by renal damage as a result of immuno-hemolytical anemia in patients who earlier have consumed the mushroom without any problems (10).

Psilocybe and other species containing halluzinogenic components like psilocin and psilocybin will not be considered here. Fatalities have not been reported after their ingestion.

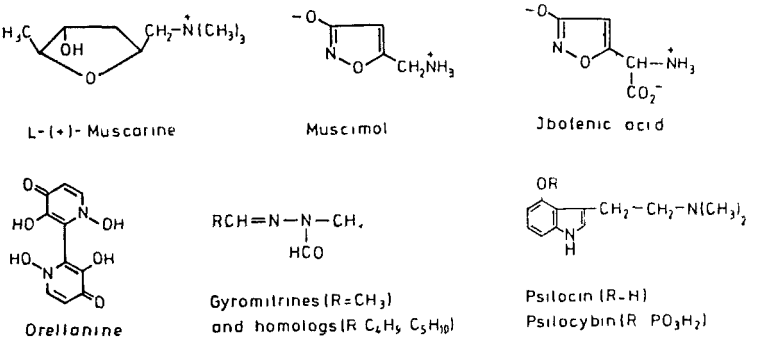
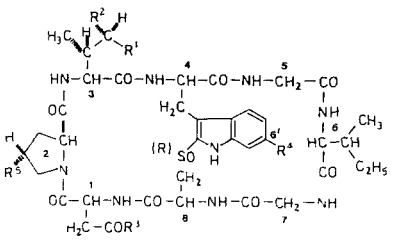


Fig. 1. Components from mushrooms, except poisonous Amanita, producing pathological events

Lethal cases reported as a consequence of ingestion of any of the mushroom species mentioned above are relatively rare. Most fatalities (more than 95%) occur with Amanita species, which accumulate amatoxins.

Poisoning by toadstools, accumulating amatoxins

Most of the fatal intoxications by mushrooms occur after ingestion of the Amanita species. The amatoxins, cyclic peptides solely responsible for those cases, have also been detected in Galerina and Lepiota species, which, although seldom, have been consumed with fatal consequences (11). The hazardous Amanita may be confused with various edible mushrooms, although for an expert, it is not difficult to recognize poisonous ones. The counsel of folklore is useless, for example, whether or not a silver spoon darkens on cooking the mushrooms, has no bearing on the edibility. However, a simple color reaction exists for α-, β-, and γ-amanitin (figure 2).



Name	R^1	R	R^3	R^4	R^5	LD_{50} (mg kg^{-1} white mouse)
α Amanitin[a]	CH_2OH	OH	NH_2	OH	OH	0.3–0.6
β Amanitin	CH_2OH	OH	OH	OH	OH	0.5
γ Amanitin	CH_3	OH	NH_2	OH	OH	0.2–0.5
ε Amanitin	CH_3	OH	OH	OH	OH	0.3–0.6
Amanin	CH_2OH	OH	OH	H	OH	0.1
Amanin amide[b]	CH_2OH	OH	NH_2	H	OH	0.1
Amanullin	CH_3	H	NH_2	OH	OH	>20
Amanullinic acid	CH_3	H	OH	OH	OH	>20
Proamanullin	CH_3	H	NH_2	OH	H	>20

[a] Systematic name Cyclic(L-asparaginyl *trans* 4 hydroxy L prolyl (*R*) 4 5 dihydroxy L isoleucyl 6 hydroxy 2 mercapto L tryptophylglycyl L isoleucylglycyl L cysteinyl) cyclic (4→8) sulfide (*R*) S oxide
[b] In *A. virosa* only

Fig. 2. Naturally occurring amatoxins

Amanita phalloides, the green death cup or "deadly agaric", "der grüne Knollenblätterpilz" grows in Central Europe from July until the end of October and is associated with deciduous trees, particularly with beeches and oaks in open forests that are not too dry. In the past in the United States, A.

phalloides was very rare, but its frequency has increased over the past 5 decades.

The mushroom develops from an egg-shaped, white "button" within 1 to 2 days to a mushroom (carpophore), reaching a height of 10-15 cm. The slightly vaulted cap develops a diameter of up to 12 cm; it is smooth, more or less deep olive-green, and often patterned with darker, radially-extending filamentous streaks. The lamellae are white; the upper stalk, which sometimes shows pale-greenish cross-stripes, is surrounded by a white cuff and the lower stalk ends in a saclike volva mostly hidden in the ground. The toxic mushroom has no specific smell or taste.

A.verna is a white variety of A.phalloides. It appears, like the former, in a similar habitat and during the same season, but, in Germany, less frequently.

A.virosa, the white "destroying angel", is now established as a species chemotaxonomically different from A.verna. It contains amatoxins, phallotoxins and, in addition, virotoxins (12, 13) (see Figure 3). Of the white mushrooms reported to occur only in North America, like A.bisporigera, A. tenuifolia, A.ocreata and A.Subalbiacea, possibly one or more are closely related or even identical to A.virosa. Comparative, chromatographic studies should be helpful in elucidating this question.

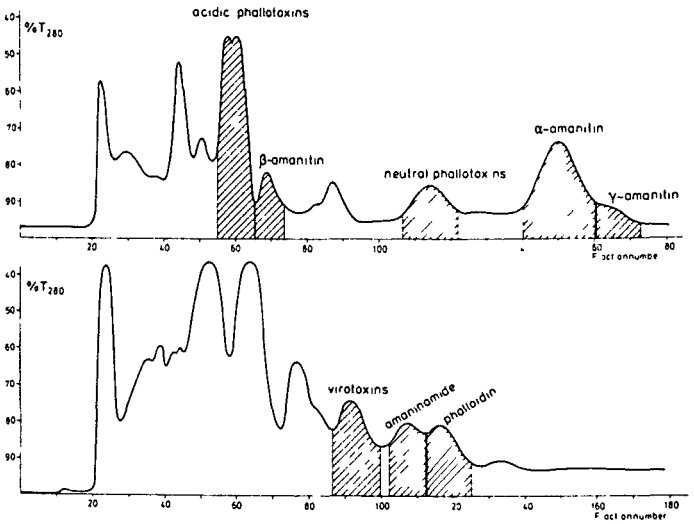
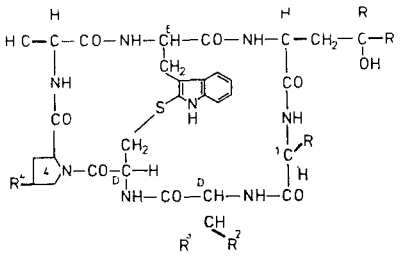


Fig. 3. UV-monitored elution diagrams of chromatography on Sephadex LH-20 in water of methanolic extracts of A.phalloides (upper line) and A.virosa.

In comparison to A.verna the carpophores of A.virosa are longer, the shape of the cap is more conical in young individuals and the stalk is lightly scutate. They are abundant in some European countries, e.g. in southern France, and are also known to occur in Scandinavia, but they are relatively rare in more central areas, since their preferred habitats are mountainous regions. Recently, they have also been found in Mexico.

Besides the disastrous amatoxins in all toxic Amanita species, a second group of less toxic substances, the phallotoxins are found, and additionally in A.virosa, the virotoxins (14), but the last two groups do not lead to lethal intoxications.



Name	R^1	R	R^3	R^4	R^5	R^6	LD_{50} (mg kg^{-1} white mouse)
Phalloin (PHN)	CH_3	CH_3	OH	OH	CH_3	CH_3	1.5
Phalloidin (PHD)[b]	CH_3	CH_3	OH	OH	CH OH	CH_3	2
Phallisin (PHS)	CH_3	CH_3	OH	OH	CH OH	CH OH	2
Prophalloin (PPN)	CH_3	CH_3	OH	H	CH_3	CH_3	>20
Phallacin (PCN)	$CH(CH_3)$	OH	CO H	OH	CH_3	CH_3	1.5
Phallacidin (PCD)	$CH(CH_3)_2$	OH	CO_2H	OH	CH OH	CH_3	1.5
Phallisacin (PSC)	$CH(CH_3)_2$	OH	CO_2H	OH	CH_2OH	CH OH	4.5

[a] R^1–R^5 see Fig 23

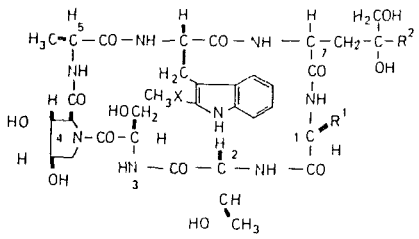
[b] Systematic name of phalloidin Cyclo(L Alanyl D threonyl L cysteinyl *cis* 4 hydroxy L prolyl L alanyl 2 mercapto L tryptophyl 4 5 dihydroxyleucyl)cyclo(3–6)sulfide

Fig. 4. Naturally occurring phallotoxins

All of the amatoxin-accumulating fungi, after ingestion, cause the same clinical symptoms, which can also be elicited in experimental animals by pure amatoxins. Human Amanita poisoning develops in four stages: a latency period (6-12 h) is of high diagnostic value because most other poisonous, but less harmful fungi, cause symptoms within 2 h after ingestion. After the lag period, a gastrointestinal phase follows, characterized by cholera-like diarrhea with concurrent dehydratation, vomiting and abdominal pains. Concomitantly, hypoglycemia develops, which can be lethal in rare cases. Although the patient feels better when the gastro-intestinal disease is over, hepatic lesions develop as determined by the serum concentration of the liver enzymes, glutamate oxalacetate transaminase (GOT), glutamate pyruvate transaminase (GPT) and lactate dehydrogenase (LDH). Together with the increase of these enzymes, blood coagulation is severely disturbed, which may induce internal bleeding. The rapid decrease in coagulation factors is, in most cases, indicative of a poor prognosis. During the final phase, the liver enzymes in the serum continue to increase. Hepatic failure can cause encephalopathy and coma. High values of creatinine and urea indicate additional damage to the kidney. In most cases, patients die in hepatic coma combined with renal failure. Death may occur as late as 6 to 8 days after ingestion of the mushrooms.

In humans, the lethal dose of amatoxins can be estimated from accidents to be about 0.1 mg/kg, or even lower. The toxins seem to be readily absorbed by the intestine. A similar situation occurs in the guinea pig, where the lethal dose is also low (0.1 mg/kg), and identical for oral, intravenous or intraperitoneal administration, indicating a strong intestinal absorption. This is surprising because other rodents, such as the mouse (LD_{50} 0.4-0.5 mg/kg), i.v.) or the rat (LD_{50} 3.5-4 mg/kg, i.v.), cannot be poisoned orally, even with high doses of amatoxins. Among other animals, cats and dogs are known to absorb amatoxins from the gut. Here, however, the intestinal absorption is slow and limits the intoxication; the oral LD_{50} dose in the dog for α amanitin is about five times higher (0.5 mg/kg) than the intravenous dose (0.1 mg/kg) and more than ten times higher in the cat.

In accordance with the capability of intestinal absorption, the gut cells of humans and dogs seem to be the first cells affected. In both species, the intestinal phase begins about 9 h after administration of the toxins. In the dog, the intestinal signs occur after oral as well as parenteral administration (15), possibly another indication for the enterohepatic circulation, which brings the intestinal cells into direct contact with the toxins.



Name	X	R^1	R^1	Percent of total
Viroidin	SO_2	$CH(CH_3)_2$	CH_3	18
Desoxoviroidin	SO	$CH(CH_3)_2$	CH_3	4
Ala^1-viroidin	SO_2	CH_3	CH_3	10
Ala^1 desoxoviroidin	SO	CH_3	CH_3	
Viroisin	So_2	$CH(CH_3)_2$	CH_2OH	49
Desoxoviroisin	SO	$CH(CH_3)_2$	CH_2OH	19

Fig. 5. Naturally occurring virotoxins

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Effective therapy of Amanita poisoning is not feasible, since the amatoxins, once they have entered their target cells, are difficult to remove from their receptor enzymes in the nuclei (RNA-polymerase B (II)). The aim, therefore, must be to reduce the concentration of toxin not yet bound to the lowest level within the shortest span of time. Obviously, the severity of intoxication depends on the amount of poison ingested and, therefore, on its concentration in the blood. Since amatoxins are excreted rapidly with the urine, radioimmunological assays (16,17) can provide information on the severity of intoxication within a few hours. Treatment should begin as early as possible to rid the patient of the amatoxins by all means: removal of stomach and intestinal contents, intake of increased amounts of charcoal, and forced diuresis. In serious cases, extracorporal purification methods, e.g. hemoperfusion over absorbents, hemodialysis or plasmapheresis may be

indicated. Excessive amatoxins are secreted from the liver with the bile into the intestine from where they are reabsorbed to affect liver cells anew etc. Interruption of this enterohepatic circulation can be obtained by charcoal and can be further enforced by various drugs, such as silymarin (18), or, less effectively, penicillin, which have been shown to inhibit the incorporation of the toxins into hepatocytes (19). Beside these drugs, other substances with unknown mechanisms (e.g. thioctic acid) have also been used, apparently with good results (20,21). In a clinical survey of 160 patients, 4 of them died, the case history of the survivors was traced over 6 months whereby the development of chronic active hepatitis was reported in several cases (22).

The entrance of the amatoxins into the liver is mediated by two proteins located in the hepatocyte membrane - the same proteins (molecular masses of 48 KD and 53 KD, respectively) which also are responsbile for the inward transport of bile acids, phalloidin, and of a series of other substances (23). As illustrated in Fig. 6 the rate of uptake of the toxin by membrane vesicles is distinctly decreased by the presence of silybin (and of other inhibitors) (24).

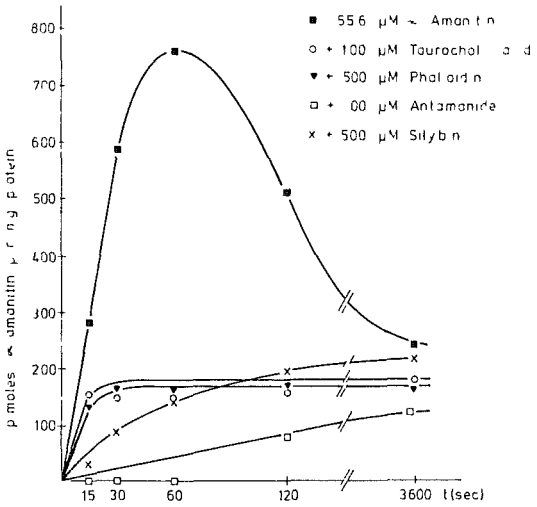

Fig. 6. Amounts of ^{3}H-labelled amatoxin in rat-liver membrane vesicles of different time intervals after addition of the drug, in the presence of a Na^{+} inward-gradient: 55.6 µM toxin alone, the same with 100 µM taurocholate, the same with 500 µM phalloidin, the same with 100 µM antamanide or 500 µM silybin (24).

The greatest safety measure is obtained by avoiding poisonous mushrooms. Nowadays a simple test exists for discriminating poisonous Amanita from edible ones: press a piece of the mushroom (cap or stalk) onto crude paper, e.g. newsprint, mark the spot with a pencil and, after drying, a drop of concentrated hydrochloric acid is applied. In the presence of almost all amatoxins, a blue color will develop within 5-10 min (24).

The molecular mechanism of amanitin intoxication.

Detailed information on the biochemical action of amanitin came from the finding of Fiume and Stirpe (25) that, in mouse liver nuclei, the RNA content decreases progressively during the first 24 h following intoxication with α-amanitin. The same authors showed that RNA synthesis in isolated liver nuclei of mice was seriously impaired after both in vivo and in vitro administration of the toxin, suggesting that amanitin inhibits the enzyme-RNA polymerase (26). In every cell nucleus reside 3 types of DNA-dependent RNA polymerases; RNA polymerase I catalyzes the synthesis of ribosomal RNAs, type II that of messenger RNAs, and type III i.a. transfer RNAs. It is the enzyme II which is inhibited by minimal amounts of amatoxins: a concentration as low as 10^{-9}M (i.e. 1 µg per liter) will inhibit the formation of mammalian mRNA on a template of DNA by 50%! One mol of the toxin binds firmly to the giant polymerase (molmass 5 x 10^5) which consequently loses its mobility along the DNA strand forming a tight tertiary transcription complex of enzyme, DNA and nascent RNA chain (27).

After cessation of mRNA synthesis the synthesis of corresponding proteins will no longer occur, depletion of proteins of the cell, mainly liver cells, will lead to necrosis in the course of several days. The lethal dose of α-amanitin for an adult human is about 10 mg per individual.

The phallotoxins and virotoxins, additional cyclic peptides from Amanita mushrooms, most probalby play no role in human mushroom intoxication. Their toxicity is much less than the toxicity of amatoxins, they are being absorbed very slowly from the intestine, if at all, and after intraperitoneal administration they will kill an animal (mouse) within 2-5 hours. They bind very strongly and specifically to F-actin. This property has been utilized in a variety of cytobiological studies which, however, are not in the scope of this presentation. For a detailed information on this area of Amanita research see Wieland (28).

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Discussion - New trends in mushroom poisoning.

A.L. de Weck

I would like to comment that amanitins have become a tool of interest to immunologists because they strongly inhibit T cell proliferation and some T cell functions as well. Immunology aside, you have mentioned that silymarin can decrease the uptake of amatoxins by the hepatocyte. Does this compound also affect the oral absorption of the toxins?

T. Wieland

Probably not. In any case, intestinal absorption of amatoxins is a very interesting topic. Man, the ginea pig, the dog and the cat absorb amatoxins from the intestine, but rabbits, rats and mice do not. On the other hand, no experimental animal absorbs phalotoxins from the intestinal tract.

F.J.C. Roe

What is the function of these toxins in the mushroom?

T. Wieland

It is not quite known. One of the current theories is that the amatoxins have a regulatory function in the transcription of RNA. Actually, amatoxins are also present, albeit in minimal amounts, in edible mushrooms. In poisonous mushrooms, RNA polymerase would be a thousand times or even less sensitive to amatoxins and they would have to be produced in much higher amounts.

O. Pelkonen

Warnings have been issued in Finland against false morel because it is felt that even after cooking some hydrazine derivatives are present in the mushroom.

T. Wieland

Yes. I cannot recommend eating these mushrooms. Sale of false morel has been forbidden in Germany for many years.

L.F. Prescott

Can you comment on the mechanism of renal damage induced by

cortinarius?

T. Wieland

It is not well known becuase of the lack of animal models.

B. Kobush

What is the sequence of events between uptake by the hepatocyte and cell death in the case of amatoxins?

T. Wieland

The first event must be binding to RNA polymerase. The late cell death is probably due to the lack of synthesis of essential proteins.