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*Übersichten*

# New Aspects of Amanita Poisoning

H. Faulstich

Max-Planck-Institute of Medical Research, Department of Natural Products, Heidelberg

**Neue Aspekte der Knollenblätterpilzvergiftung**

**Zusammenfassung.** Amatoxine finden sich im grünen und in den weißen Knollenblätterpilzen, aber auch in anderen Pilzarten. Diese Gifte sind die alleinige Ursache der tödlich verlaufenden Knollenblätterpilzvergiftung. Die charakteristische, lange Latenzzeit der Vergiftung ist einerseits ein zuverlässiges Mittel zur Diagnose, fordert aber andererseits eine sofortige Behandlung, um die Toxinkonzentration im Serum rasch zu senken und damit die Zeit abzukürzen, in der z.B. die Leberzellen den Giften ausgesetzt sind. In letzter Zeit wurden mehrere biologische Assays für Amatoxine beschrieben, deren Nachweisgrenze bei etwa 0,5 ng/ml liegt. Mithilfe solcher Assays kann die Schwere einer Knollenblätterpilzvergiftung bestimmt werden, eine wichtige Voraussetzung zur Auffindung der optimalen Therapie.

Amatoxine zerstören eukaryotische Zellen durch Hemmung der Transkription von m-RNS. Betroffen sind vor allem Leberzellen, die selbst bei geringer Toxinkonzentration geschädigt werden. Leberzellen nehmen das Gift relativ schnell auf, scheiden es jedoch auch rasch wieder mit der Galle aus. Dementsprechend muß man Maßnahmen treffen, um bei der menschlichen Vergiftung den enterohepatischen Kreislauf zu unterbrechen. Obwohl Amatoxine auch durch die Niere schnell ausgeschieden werden, kann eine extrakorporale Reinigung des Blutes angezeigt sein, z.B. zur schnellen Entfernung der Gifte aus dem Kreislauf oder um die Niere nicht zu lange den Giften zu exponieren. Diese Übersicht berichtet über aktuelle Methoden zur Blutreinigung, über Chemotherapeutika zur Behandlung der Knollenblätterpilzvergiftung, sowie über den Einsatz von Faktoren zur Leberregeneration. Alle Therapiemaßnahmen, die sich entweder bewährt haben oder nach neueren Erkenntnissen empfohlen werden können, sind in einer Liste zusammengefaßt.

**Schlüsselwörter:** Amatoxine – Pilzvergiftung – Diagnose und Therapie – extrakorporale Reinigung – enterohepatischer Kreislauf

**Summary.** Amatoxins occur in the green and white Amanita species, but also in other mushroom genera. These toxins are the sole cause of fatal human Amanita intoxications. Their long latency period is a useful tool for diagnosing such cases of poisoning, but on the other hand necessitates an immediate treatment in order to rapidly decrease the toxin concentration in the serum and to shorten the time of exposure of e.g. liver cells. Various bioassays for amatoxins were reported having a limit of detection near to 0.5 ng/ml; they have become useful tools to determine the severity of an intoxication, a prerequisite for finding the optimum therapy.

Amatoxins damage eukaryotic cells by inhibiting their transcriptional process. Lesions are found particularily in the parenchymal cells of the liver, even at low toxin concentrations. These cells incorporate the toxins relatively fast, but at the same time excrete them rapidly into the bile. Accordingly, in human Amanita poisoning the enterohepatic circulation must be interrupted. Although the amatoxins easily undergo ultrafiltration in the kidney, extracorporal purification methods may be indicated for reasons such as rapid clearing of the serum or shortening the time of kidney exposure to the toxins. This review will report on extracorporal purification methods, on drugs used for chemotherapy, as well as on factors capable of inducing liver regeneration. All procedures for acute Amanita poisoning, either proved or suggested, will be listed.

**Key words:** Amatoxins – Mushroom poisoning – Diagnosis and therapy – Extracorporal purification – Enterohepatic circulation

Nearly all fatal human mushroom casualities are caused by only a few Amanita species. In Europe, the green death cap, Amanita phalloides, is the most common of these deadly poisonous mushrooms. The white species, Amanita virosa, is only rarely seen, however, it prevails in the United States, along with some other toxic Amanita species unknown in Europe. Only recently, an excellent review on all toxic Amanita species has been published by Seeger and Stijve [45]. However, the toxic components causing the typical symptoms of Amanita poisoning are not restricted to the Amanita species. They have been found in other mushrooms too, namely in small Lepiota and in Galerina species, the latter, for example, being small brown mushrooms that grow on wood. Only recently, two cases of serious poisoning with Galerina autumnalis have been reported in the United States [6].

## The Amatoxins

The compounds which cause the fatal effects are a group of cyclic peptides, the amatoxins. Their concentration in Amanita phalloides has been determined to be 0.2 to 0.4 mg per g fresh tissue [23, 7]. The structures of all amatoxins have been elucidated by Wieland and his laboratory in the past years [53]. There are only small differences in their structures as well as toxic activities [52]. Amatoxins inhibit the transcription of DNA to RNA. As a consequence, protein synthesis is blocked, followed by necrosis of the affected cells.

During the First International Symposium on Amanita Toxins and Amanita Poisoning held at Heidelberg in November 1978 [1], there was no controversy concerning the conclusion that in fatal cases of Amanita phalloides intoxication, the amatoxins are the sole cause of the fatality. A second group of toxic peptides present in the green death cap, the phallotoxins, obviously do not contribute to human poisoning. Therefore, the above Symposium was concerned with the nature and the activities of only the amatoxins.

## Analytical Methods

The most commonly used method for the detection of amatoxins is the reaction with cinnamic aldehyde and hydrochloric acid yielding a bright violet color. In combination with high performance thin layer chromatography the limit of detection by this colorimetric method is as low as 50 ng of amatoxins [46]. In an analogous reaction making use of lignin-like aldehydes present in crude papers, Wieland found a simple procedure to separate deadly poisonous mushrooms from edible ones. He developed the crude mushroom juice with 8 N hydrochloric acid on newspapers, where the amatoxins give a prussian-blue color [50]. This rapid assay may be used to detect amatoxins in all deadly poisonous mushrooms including those with lower amatoxin content like the Galerina species. However, no colorimetric method is sensitive enough to detect the low concentrations of amatoxins present in the serum of patients.

Many of the data discussed below became accessible only by improved analytical methods for amatoxins. The discovery of amatoxins in the serum or urine of a patient has been achieved by radioimmunoassay [10, 24, 25, 31]. Antibodies against protein-bound amatoxins have been raised in the rabbit and the rat. Both types of immunoglobulins bind free amatoxins with high affinity ($K_D \sim 10^{-9}$M) permitting the determination of amatoxin concentrations down to 0.5 ng/ml. Due to the extremely high toxicity of the protein conjugates [11] used as antigens, only small amounts of the antibodies have been produced. The amount is, thus far, insufficient to be utilized in a therapeutic treatment of Amanita poisoning, even in experimental animals.

Most recently, in vitro assays with human lymphocytes have also been proposed for analytical use. Low concentrations of amatoxins in human serum were shown to be able to inhibit [$^3$H]-thymidine incorporation into lectin-stimulated cells [8]. Likewise, amatoxins reduced the E-rosette formation of T-Lymphocytes with sheep erythrocytes [41].

## Non Specificity of Amatoxins

Amatoxins are often regarded as hepatotoxic agents. However, they exhibit their toxic activities in all kinds of eucaryotic cells, provided they can penetrate them. Penetration into liver parenchymal cells is rapid as compared to many other body cells or to cell lines in culture. Another reason for the apparent specific toxicity to liver cells may be that cells with a high rate of protein synthesis like hepatocytes are more susceptible to amatoxins than those with lower rates.

Cells which must be assumed to be easily penetrated by amatoxins are those of the intestinal epithelium. Lesions in these cells represent the first symptoms of Amanita intoxication. Damage of the epithelial cells obviously does not require intestinal contact with the toxins, since in experiments with dogs, gastrointestinal symptoms could also be produced by parenteral administration of $\alpha$-amanitin [20, 32].

In liver parenchymal cells the predominant lesions are depletion of glycogen, fatty degeneration and alterations of nuclei. In the end these cells become necrotic leading to the symptom of hepatic failure. Sinusoidal cells of the liver are not affected. In kidney cells irreversible lesions were observed in a rather late phase of intoxication and only at low doses of amatoxins. This was found to be true for the white mouse [33] as well as for the dog [29]. In both species high doses of amatoxins produce other lesions that lead to death before renal failure can develop (hepatic failure and hypoglycemia, respectively).

Other parenchymal cells exhibiting lesions after amatoxin treatment are pancreatic cells [16] and cells of rat testis, as reported recently [47]. Of the blood cells lymphocytes can be affected as shown by the two bioassays mentioned above. Finally, macrophages and other protein consuming cells can be killed by amatoxins provided these are conjugated to proteins. The lesions in macrophages are very similar to those obtained by free amatoxins in other cells and, most probably, the amatoxins are released from the protein by proteolytic enzymes inside the cells. Since macrophages completely resist the native amatoxins, the example provides further evidence that in fact the penetration rate of the toxins into cells determines the lesions they cause.

## Symptoms

Symptoms of human Amanita poisoning develop in 4 stages [43]: 1. A period of latency (6–12 h); 2. the gastro-intestinal phase (12–24 h); 3. a second latency phase with an apparent and delusive recovery of the patient and 4. the hepatic phase.

The latency period of 6 to 12 h is unique for amatoxin poisoning. It is of high diagnostic value, because most other poisonous but less harmful fungi cause symptoms within 2 h after ingestion. However, gastrointestinal symptoms within 2 h are not an unambigous proof for the absence of deadly poisonous Amanitas, since many people collect mixed mushrooms which may contain poisonous species producing early symptoms and amatoxin containing species as well.

The gastrointestinal phase is characterized by cholera-like diarrhea with concurrent dehydration, vomiting and abdominal pains. Concomitantly, hypoglycemia develops, which in experiments with dogs proved to be lethal in most cases. In man the extent of hypoglycemia is unknown, while for decades the treatment of Amanita phalloides intoxication with glucose infusion has become an obligatory part of therapy.

Although the patient feels better when the gastrointestinal disease is over, the hepatic lesions develop as monitored by the serum concentration of SGOT, SGPT and LDH. Together with the rise of these enzymes, the blood coagulation is severely disturbed, which may give rise to internal bleedings. A rapid fall of 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 an additional damage of the kidney cells. In most cases patients die in hepatic failure combined with renal failure. Death may occur as late as 6–8 days after ingestion of the mushrooms.

## Dynamic Aspects of Liver Lesions

Pharmacodynamic studies with amatoxins were performed in the perfused rat liver, a model which may be different from human liver. As for the toxin, [$^3$H]O-methyl-dehydroxymethyl-α-amanitin had to be used [51]. It is 15 times less toxic in the white mouse than α-amanitin but does not undergo microsomal oxidation in the radiolabel as other, more toxic, radioactively labelled amanitin derivatives do [18]. The results, uncertain as they may be, provide the first information on the pharmacodynamics of amatoxins in the liver [36].

We found that within a wide range of toxin concentrations in the perfusion medium ($10^{-6}$ M to $10^{-8}$ M) the uptake of amatoxins was proportional to the serum concentration. Since the liver excreted about 60% of the incorporated toxin in the bile, the toxin concentration inside the liver reached a steady state of about 0.4 times the concentration in the perfusion medium. Taking into account the low concentration ($5 \times 10^{-8}$ M) of the target enzymes, the DNA-dependant RNA-polymerases II (or B), [14] a 15 min perfusion of the rat liver with a toxin concentration as low as $3 \times 10^{-7}$ M is sufficient to completely block RNA-synthesis in hepatocytes. This is in good agreement with the observation that even very low serum concentrations of α-amanitin may cause cellular lesions in the livers of dogs [10].

As already stated above, the rat liver is a poor model. In the pig liver, we found yet a higher uptake of the same amatoxin even when the toxin passed the liver only once after an intraportal injection [30]. No pharmacokinetic data is available for the human liver, however, it may be expected to be closer to pig liver than to rat liver.

In any case, the most important fact seems to be that, in general, toxins are excreted into the bile to a great extent, and that the liver has the chance to rid itself of them, provided further penetration into the liver is stopped. The conclusion to be drawn from these studies is a dynamic picture of amatoxin activity in liver, where the most decisive parameter is the time during which the nuclei of the liver cells is exposed to amatoxins, even at low concentrations.

## Enterohepatic Circulation of Amatoxins

The excretion of amatoxins via the bile has consequences for the course of intoxication. In animal species which absorb the amatoxins in their intestinal tract, a recycling of the toxins can occur. This entero-

hepatic circulation prolongs the presence of amatoxins in the serum and, consequently, the period over which the liver cells are exposed.

There exist laboratory animals like the rat and the mouse, which do not absorb the toxins in the gut. Accordingly, these animals species cannot be poisoned by oral administration even of very high doses of amatoxins. It is known that mice in the wild will occasionally nibble from deadly poisonous Amanitas, probably without suffering any harm. For these species, the enterohepatic circulation is of no significance.

Other animals like the dog can absorb the toxins in the intestinal tract. Here, interruption of the enterohepatic circulation results in the dogs surviving absolutely lethal doses of an amatoxin. This was first demonstrated by applying a bile fistula to Beagle dogs [27] and has recently been confirmed by Vogel [49], who conclusively showed that in the dog, silymarin exhibits its long-lasting antitoxic activity by interrupting the enterohepatic circulation. Lethal poisoning of the dog by oral administration of amatoxins needs, however, a dose 7 fold greater than that required by parenteral administration. This indicates a rather slow absorption of the toxins in the intestinal tract of the dog. Hence we can expect a significant but moderate participation of enterohepatic circulation in poisoned dogs.

The situation is different in animals like the guinea pig, which absorb amatoxins so rapidly that the $LD_{50}$ values of enteral and parenteral application are identical (0.1 mg/kg body weight) [26]. For humans no pharmacokinetic data are available, but much evidence indicates that in man the situation must be similar to that in the guinea pig. Only recently, it has been reported that amatoxins were detected in human duodenal juice [10]. Therefore, enterohepatic circulation should be seriously taken into account in human Amanita poisoning. Application of charcoal, used thus far to prevent the primary absorption of the toxins is, of course, also useful for the interruption of the enterohepatic circulation. A more reliably method, however, seems to be the insertion of a tube according to Bartelheimer et al. [2], which makes possible the continuous aspiration of the bile fluid immediately after entering the duodenum.

The importance of enterohepatic circulation is also confirmed by the observation that three drugs which were claimed to possess antitoxic activity in man, in fact inhibited the penetration of amatoxins into rat liver cells [36]. If silymarin, penicillin and prednisolon have a similar effect in liver of man, they would concomitantly inhibit the enterohepatic circulation of amatoxins. This would justify the use of these drugs in therapy of human Amanita poisonings initially, and also beyond the time of primary intestinal absorption.

## Excretion of Amatoxins in Urine

Amatoxins have a molecular weight of about 900 daltons, and accordingly, the toxins are easily dialysable. There are no data available on clearance values in humans so far, however, in in vitro systems with synthetic membranes, the amatoxins were dialysed only about 3–4 times slower than were salts [19].

Correspondingly, the excretion of the circulation toxins by urine is a very rapid process. For example, after the injection of lethal doses of a radioactively labeled amatoxin into a dog, it took only 5 h to decrease the serum concentration to below the limit of detection ($<0.3\%$ of the dose administered) [22]. These figures were found to be different for oral poisoning, where in the dog the serum concentration was kept up by continuing intestinal absorption [28].

The high excretion rate as well as the fact that urine reflects the situation in the serum several hours before in a manifold concentrated state, makes urine sampling of patients of high diagnostic value. In fact, in a group of more than 50 poisoned persons, amatoxins were detected by radioimmunoassay in the urine of all patients by an Italian team [37]. This shows that the investigation of urine samples is an appropriate method for diagnosis even in mild cases of Amanita poisoning.

Amatoxins also cause cellular lesions in kidney cells [33]. In amanitin-poisoned dogs which had died by renal failure, necrosis of epithelial cells was described, however, in the proximal tubules only. This suggests that amatoxins may penetrate the kidney cells during a process of either reabsorption or secretion. Secretion, however, could be ruled out by the measurement of amanitin clearance in the dog, which proved to be lower than that of $^{51}$Cr-EDTA [21].

## Coagulation Disorder by Amatoxins

In addition to the lesions in liver and kidney cells another dominant symptom is the coagulation disorder. It has been observed in many experimental animals as well as during human Amanita poisoning. Some dogs were observed to die in a coma different from that induced by hypoglycemia or hepatic failure, probably caused by hypoxemia [29]. Also in man, a few fatal cases by hemorrhage have been reported.

It is reasonable to assume that a block in protein synthesis and the damage of liver cells must cause coagulation disorders due to a deficiency of coagula-

tion factors. This may be true, however, there are indications that consumption is particularily involved in the coagulopathy. This has been concluded from the observation that there is a simultaneous decrease of clotting factors with different turnover rates [54]. During the Symposium, it was a matter of controversy as to whether heparin should be administered in order to prevent disseminated intravascular coagulopathy.

## Molecular Mechanism of Amatoxin Activity

By the investigation of cellular lesions in mouse liver cells exposed to α-amanitin, Fiume and Stirpe [34] detected the molecular mechanism of amanitin toxicity. They observed that the first structural alterations occurred in the nuclei of the cells long before any structures in the cytoplasm were changed. Persuing this trace they detected that RNA-synthesis was decreased by amatoxins.

Today we know that RNA is synthesized by at least 3 classes of DNA-dependant RNA polymerases. RNA-polymerases I (or A) are located in the nucleoli; these enzymes synthesize ribosomal RNA and are completely resistant to amatoxins. RNA polymerases II (or B) are located in the nucleoplasm; they transcribe the precursors of messenger-RNA and are highly sensitive to amatoxins ($K_i \sim 10^{-8}$ M). Finally, a third class, the RNA polymerases III (or C) transcribe transfer RNA and other low molecular weight (5 S) RNA. These enzymes are again sensitive to amatoxins, but only at high doses ($K_i \sim 10^{-5}$ M). For the understanding of the lesions in liver cells it may by important to know that in mouse liver the RNA polymerases I, although being totally resistant to amatoxins in vitro, are inhibited in vivo. There is no clear cut explanation for this so far. The observation however, may be another reason for the preference of amatoxin lesions in liver cells.

The molecular interaction of the toxic peptides with RNA polymerases II has been excellently investigated by Cochet-Meilhac and Chambon [13]. These workers confirmed that the toxin forms a 1:1 complex with the enzyme and determined the various thermodynamic and kinetic constants of this complex. Among the different subunits of RNA polymerase II, the polypeptide chain with a molecular weight of about 140.000 was pinpointed by Brodner and Wieland to bind to the toxin [9]. By comparison of the thermodynamic and kinetic data of the enzyme complexes of several amatoxins with their in vivo toxicity, it was found that toxicity is predominantely determined by the dissociation rate constant [13]. This provides additional evidence for the importance of dynamic aspects in amatoxin poisoning.

For a more detailed presentation of the progress made in biochemistry of the amatoxins the reader is referred to two recent reviews [18, 52].

## Serum Concentration of Amatoxins

As concluded from the pharmacodynamic data found in the perfused rat liver, the serum concentration of amatoxins directly determines the intracellular concentration in, e.g. hepatocytes, and most probably, also in other cells. Since we know that even a low amatoxin concentration in the serum is sufficient to block the entire RNA polymerase B activity in the heavily exposed liver cells, the crucial parameter is the period of time that the cells are exposed to the toxins. Toxin concentration in serum is of high importance because it determines that period of time in different ways.

A shown in Fig. 1, amatoxin concentration in the serum is dependant upon several processes. It is kept up by absorption of toxins in the intestinal tract which perhaps even continues to some extent after careful washings of the bowel. Already, soon after ingestion of the mushroom the intestinal absorption includes part of the toxins which have passed the liver and were excreted by the bile. The kidney eliminates the major quantity of the toxin from the serum. However, there are indications that the kidney also reabsorbs part of the toxins. If this is confirmed, it will be advantagous to further increase the renal elimination by means of a forced diuresis. Finally, it is yet undetermined whether particularily during a later phase of poisoning, the serum concentration can be raised by a back flow of toxins from other organs. For example, after oral poisoning of the dog the serum concentration was found highest 1.5 h after ingestion [28]. During this time the toxins may also penetrate into other tissue cells which are greatly insensitive to the toxin activities, e.g. muscle cells. Such cells may, later on, slowly release the pooled toxins into the serum.

## Extracorporal Purification of Blood

The nature of the amatoxins provides a very good opportunity for the application of extracorporal purification methods like hemodialysis, hemofiltration or hemoperfusion. Amatoxins are easily dialysable and as well possess a high affinity to surfaces of charcoal or certain polymers used as filling material of hemoperfusion cartridges. Therefore, there is no doubt of the efficacy of these methods. Controversy about their application may, however, arise from the question

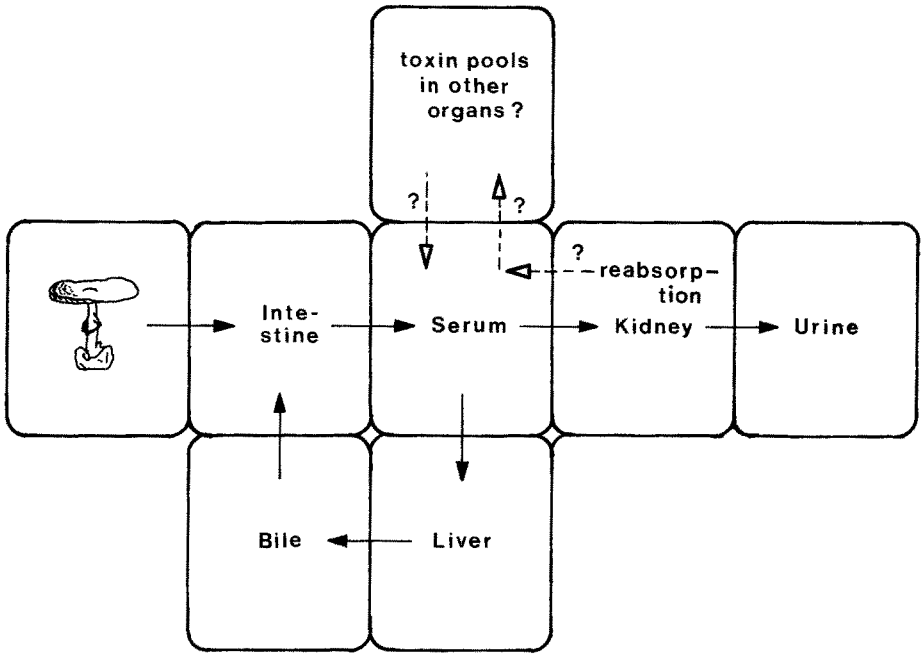


**Fig. 1.** Kinetics of amatoxins during Amanita poisoning

as to whether at the time of hospitalisation, the pool of toxins in the blood of a patient is still high enough to justify an extracorporal clearification procedure.

Considering the data presently available, the answer to this question must be positive in cases when the patient arrives at the hospital within the first 2 days after ingestion of the toxins. The most serious arguments come from the observation [10] that low concentrations of toxins could be detected in sera of patients even 36 to 48 h after ingestion. Such low serum concentrations of amatoxins are possibly maintained, even after careful washing of the bowel. Processes contributing to this late serum toxin concentration may be either absorption of toxin from residues left in the intestine, from enterohepatic circulation, or by toxin reflux from other organs.

As discussed above, diuresis, especially forced diuresis is very efficient in eliminating amatoxins. However, another point of consideration indicating extracorporal clearing instead of e.g. forced diuresis could be that by extracorporal methods the elimination of the toxins can be achieved without exposing the kidney cells. As a result, cellular lesions of kidney cells may be restrained. Independant of an indication by the presence of toxins in the serum, there may be another indication for extracorporal purification if an hepatic encephalopathy develops.

Among the different methods of extracorporal purification hemoperfusion over polymeric adsorbents was most effective in removing the amatoxins [38]. Hemofiltration proved to be of similar effectiveness in these in vitro studies and is combined with a very low risk of complications in praxi. An effect comparable to the different extracorporal purification methods may have plasmapheresis, which has been used occasionally during Amanita poisoning.

Although there were reservations on the usefulness of late hemoperfusion for the elimination of hepatoxic substances, since it did not improve the survival rate [15], the majority of the Symposium favored the opinion that early treatment with either of the methods would help save lives. A conclusive evaluation of the benefit of extracorporal purification methods is, however, thus far impossible. In all cases the method used was in combination with other treatment or drugs; therefore, the success of such therapy could not be definitely attributed to the extracorporal purification method alone.

## Liver Regeneration

One process which so far has been completely disregarded in the treatment of Amanita poisoning is that of liver regeneration. By the exposure to amatoxins the major portion of liver parenchymal cells may become necrotic. In this situation the proliferation of the residual hepatocytes probably decides if a patient will live or is going to die. Therefore it is of great importance at what time and to what extent the proliferation of liver cells begins.

Initiated by Usadel, the regeneration of liver cells was studied in fatally poisoned rats [48]. Spontanous

proliferation was found to be very low; only 1.3% of the hepatocytes incorporated [$^3$H]thymidine as a marker for DNA synthesis 2.5 days after poisoning. This rate could be enhanced several times by treatment with hormones or with a growth factor. For example, by frequent administration of insulin plus growth hormone DNA synthesis was active in 10.2% of the cells. Administration of the tripeptide glycylhistidyllysine, a liver cell growth factor first described by Pickart and Thaler [42, 44] enhanced the portion of DNA synthesizing cells to 14.1%. Simultanously, the survival time of the poisoned rats was prolonged significantly.

These results provide some hope that in the future physicians will be able to actively stimulate the repair processes of an amatoxin-damaged liver. However, while the liver becomes restored, it may occur that another one of the wide-spread lesions caused by the toxins becomes decisive for the patient. Only further studies can show whether hormone induced liver regeneration can provide a real improvement for the prognosis of the patient.

## Chemotherapy

In the past many drugs have been claimed to possess antitoxic activities in Amanita poisoning, among them penicillin, neomycine, cytochrome c, predinsolone, thioctic acid, silymarin, and even high doses of vitamin C.

Cytochrome c has been suggested for human therapy by Floersheim since the protein protected the white mouse from lethal doses of amatoxins even if administered 8–12 h after the toxin [35]. Penicillin showed a similar but less pronounced activity when used alone, but was synergistic when used in combination with cytochrome c. The mechanism by which cytochrome c may act is completely unknown. For penicillin it could be established that high concentrations of the antibiotic inhibited the penetration of amatoxins into the perfused rat liver [36]. A similar effect in the perfused rat liver was detected for prednisolone which, however, unlike cytochrome c and penicillin, protects the white mouse only if administered before or together with α-amanitin.

Silymarin is a mixture of biologically active flavones found in the milk thistle. The drug has been suggested for amatoxin therapy by Vogel on the basis of its antitoxic effects in mice, rats and dogs [49]. One of the components of silymarin, silybin, proved to be the most potent inhibitor found thus far for penetration of amatoxins into liver cells of the rat. Provided this inhibitory activity is not confined to rats, the component would be capable of blocking both the primary absorption as well as the enterohepatic circulation of the amatoxins in mammalian liver. Recently, silybin has been also shown to induce severalfold the synthesis of ribosomal RNA in rat livers [40] which is blocked by amatoxins. These biological activities recommend silymarin as a promising agent for Amanita poisoning.

Neither the molecular mechanism nor antitoxic activities in any experimental animals were reported for thioctic acid. This agent was introduced into human Amanita therapy by Kubička in 1959. Since then it has been widely used, particularily in Czechoslovakia, Hungaria, Italy and in the United States. Mortality statistics of Amanita casualties seem, in fact, to provide evidence for a therapeutic value of this drug. The mortality rates under thioctic acid treatment were 14% of 20 patients [17]; 10% of 34 patients; 11% of 75 patients [3]; and 0% of 4 patients [5]. All mortality rates are significantly lower than the approximate 30% mortality which are generally accepted for cases of Amanita poisoning. However, such statistics are not reliable, because in all cases the severity of the intoxication was unknown. The grade of intoxication has so far only been estimated by anamnestic data, which are usually poor, and by determinations of serum enzymes and metabolites which indicate cellular damages of liver or kidney but may not be directly related to the grade of intoxication. Only recently, more reliable analytical data became available by direct measurement of amatoxin concentrations in the urine, serum, and duodenal juices of patients [24, 31]. Such analyses were not, at the time, available for the thioctic acid statistics. Although affected with this uncertainity, the favorable statistics of thioctic treatment should not be disregarded but rather be taken as a challenge for experimental investigation in order to detect the possible mechanism of action of this drug.

On a poor experimental basis rest the components used in a therapy proposed by Bastien [4], such as neomycin, nitrofuroxazide and high doses of vitamin C. This chemotherapy has been used in several hospitals of eastern France, also in combination with Soludactone [39]. The mortality statistics seem likewise to be favorable, 13% of 39 patients, but are of course uncertain for the same reasons noted above. However, in criticizm of an improved chemotherapy one has to consider that so far nobody can rigorously exclude that a drug claimed to possess antitoxic effect may influence one of the many processes in Amanita poisoning, which have by no means been thoroughly investigated thus far.

## Conclusions

Similar to many other toxins and antibiotics, the amatoxins cannot damage cells directly. No fatal lesions are known in cells which were only in a short contact

with amatoxins. On the contrary, amatoxins inhibit an important vital process, the transcription of DNA to messenger RNA. It is by the impediment of this transcriptional process that most lesions caused by the amatoxins can be understood. This is true for early structural alterations in the nucleus like chromatin segregation but also for late lesions occuring in cell organelles and membranes. After being exposed to amatoxins, cells suffer from a deficiency of transcriptional information, which first must lead to a lack of essential proteins and, finally, to cell necrosis.

By these considerations it becomes evident that the crucial factor for a cell survival must be the period of time the amatoxins were present inside the cell in a concentration sufficient to inhibit the transcriptional process ($>10^{-8}$M). This period of time, after which irreversible lesions in a cell will occur is unknown and may be different for the different cell species. The penetration rates and the excretion rates of amatoxins for the various cells, which determine their specific sensibilities to amatoxins may likewise be dissimilar. In any case, however, the crucial period of an inhibitory intracellular amatoxin concentration must depend on the concentration of amatoxins in the extracellular medium. This rationale makes it obvious that the serum concentration of the toxins plays a central role for the damage of the various cells in the body.

Accordingly, the most prominent aim of Amanita therapy must be to decrease the serum concentration of amatoxins as soon as possible. Many of the treatments discussed above were established to serve this purpose. After washing of the bowel, either forced diuresis, plasmapheresis or one of the extracorporal purification methods should be applied. Simultaneously, the bile fluid should be removed. All these treatments should be performed in a fight against time. It may well be that a critical level of amatoxins sustained in the serum for only as short a period as one more hour can determine the chance of survival of a patient.

In accordance to this, the time of hospitalisation is of high importance. It is evident that all treatments directed towards a decrease of toxin serum concentration must be more effective the earlier they were started. In the past, unfortunately, patients often arrived at the hospital 48 h after the fatal dish or even later. Therefore, mushroom gatherers must be informed and educated. Likewise, practioners and small hospitals should be obliged to routinely ask for the possibility of a mushroom poisoning, if consulted by patients with severe abdominal disorders in summer or autumn. If the latency period is determined to be longer than 6 h, the patient should be immediately transfered to a clinic prepared to treat such cases by following an established and well organized protocol. Many contributors of the last Symposium agreed that mortality was higher, if the treatments used were started late. We must learn that Amanita poisoning is a case of emergency, comparable to acute kidney failure.

One last point to be mentioned is the analytical control of Amanita patients. If the time of ingestion is known and mean values of amatoxin clearance in man has been established, it should be possible to estimate the incorporated toxin amount by determining the toxin serum concentration at the time of hospitalisation. Such a common denominator is the prerequisite for any valid mortality statistics and also the basis for any evaluation of the different therapeutic treatments and drugs, in order to determine the optimum therapy.

## Suggested Procedures for Diagnosis and Therapy

*Diagnosis (Arbitrary)*

Obtaining of urine and serum samples immediately after hospitalisation of the patient for amatoxin analysis.

*Therapy (Obligatory)*

1. Immediate washing of stomach and intestine (during the first 36 h after the meal). Addition of charcoal to bind amatoxins in the gastrointestinal tract.
2. Rehydration. Restoration of water balance and electrolytes.
3. Removal of amatoxins from the blood (during the first 48 h after the meal) by either forced diuresis, hemofiltration, hemoperfusion using cartridges with polymeric adsorbants, or plasmapheresis.

*Therapy (Recommended)*

1. Glucose infusion in severe cases where hypoglycemia is impending.
2. Chemotherapy with thioctic acid (100–300 mg/day) or silymarine or penicillin in high doses.

*Therapy (Under Clinical Investigation)*

1. Immediate insertion of a duodenal tube according to Bartelheimer et al. [31] to remove the bile fluid. The duodenal juice may be kept for amatoxin analysis.
2. Induction of liver cell proliferation by i.v. administration of 1 U/h insulin and 40 U/h growth hormone [48].

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Priv.-Doz. Dr. H. Faulstich
Max-Planck-Institut für
Medizinische Forschung
Abt. Naturstoff-Chemie
Jahnstr. 29
D-6900 Heidelberg
Federal Republic of Germany