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Histological Criteria for Diagnosis of Amanita Phalloides Poisoning

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ABSTRACT: Five fatal cases of poisoning from ingestion of Amanita phalloides, a very common mushroom in central Italy, are reported. The fact that four of the cases occurred simultaneously enabled uniform collection of clinical, pathology and toxicology data, which is presented with particular emphasis on the histological aspects. The fifth case involved a six-year-old girl, and is discussed with reference to differential diagnosis with respect to Reye's syndrome, which was the initial diagnosis, demonstrated incorrect by the histology, pathology and toxicology findings.

The typical liver and kidney alterations of Amanita phalloides poisoning, consisting of massive hepatic central lobular cell necrosis and acute tubular necrosis of the kidney are described. Outside the liver, there was often general hemorrhagic diathesis and severe brain edema.

Although poisoning by Amanita phalloides is rare, these cases confirm the requirement for as complete a comparison as possible between circumstantial histopathological and toxicological data for the purposes of forensic diagnosis.

KEYWORDS: forensic science, forensic medicine, mushroom poisoning, Amanita phalloides, liver damage, kidney damage, autopsy

Cases of mushroom poisoning are quite frequent in the wooded areas of central Tuscany, in Italy (1,2,3,4). A common culprit is Amanita phalloides, member of the widespread, mostly edible genus Amanita (5,6). The small number of fatal cases are thanks to the recent availability of many excellent publications on the subject of wild mushrooms.

We report five cases recently studied from the clinical, histopathological and toxicological points of view in the Department of Forensic Science of the University of Siena. The diagnostic protocols used could be helpful for medical examiners investigating similar fatal cases (1).

The main characteristics of Amanita phalloides, a mushroom that grows in summer and autumn, are its high toxicity (20–50 g of fresh mushrooms can cause severe liver and kidney damage) due to a series of low molecular weight polypeptides (amatoxins, phallotoxins, viroisin, viroidin), the most lethal of which are the amatoxins (α-amanitin, β-amanitin, γ-amanitin, ϵ-amanitin, amanin, o-methyl-α-amanitin and amanullin) which consist of ectopeptides that bind (α-amanitin) extranuclear RNA-polymerase B, inhibiting it so that protein content decreases and cell necrosis sets in (7). Viroisin, viroidin and the phallotoxins (phalloidin, phalloin, phallacidin, phallin B, phallisacin, phallacin) seem to be of minor toxicological importance; they consist of heptapeptides with rapid action but are generally regarded as inactive when taken orally. Amanitin poisoning is characterized by the four following distinct clinical stages.

1. Incubation stage, usually 8 to 12 hours after ingestion (range: 6–16 h).
2. Gastrointestinal stage. Recurring episodes of abdominal pain, vomiting and diarrhea, which may be followed by severe electrolyte imbalance, dehydration, tachycardia and shock, which if not treated promptly may lead to acute renal failure and death.
3. Cytotoxic stage, 24–48 h after ingestion, there are clinical and biochemical signs of liver damage often progressing rapidly.
4. Comatose stage. Prothrombin values can drop below 10% and blood levels of bilirubin and ammonia are high. After this period, generally on the 4th or 5th day, there are two clinical outcomes: a) slow resolution with a decrease in enzyme levels and recovery of prothrombin activity; or b) an increase in ALT and AST levels and persistently low prothrombin levels, both of which are indices of massive liver necrosis. The prognosis is grave. Aminoacidemia increases and hepatic coma occurs with convulsions and respiratory failure, complicated by profuse hemorrhagic diathesis.

Case Reports

We report five cases of amanita poisoning undergoing autopsy in our Department. All the autopsies were performed within 24 hours of death. The first four cases involved victims aged 2 years, 5 years, 8 years and 65 years who had the same typical Amanita phalloides poisoning symptoms manifesting after an incubation period of 6–8 h. Mushrooms considered to be edible were picked during a trip to the mountains. They each ate two or three mushrooms. Two days after consuming the mushrooms they developed the following symptoms: vomiting, often uncontrollable, abdominal pain and mucosanguineous diarrhea, almost like that of cholera (gastrointestinal stage). All four subjects were rushed to hospital. These symptoms were soon followed by signs of acute renal failure, and shortly after, despite therapy, by massive liver necrosis and renal tubular damage (cytotoxic stage).

The following clinical data were recorded in all cases: jaundice, hepatomegaly, hemorrhaging due to inadequate liver synthesis of clotting factors, oligo-anuria due to acute renal failure, and cerebral involvement. Blood chemistry (given in Table 1) showed severe

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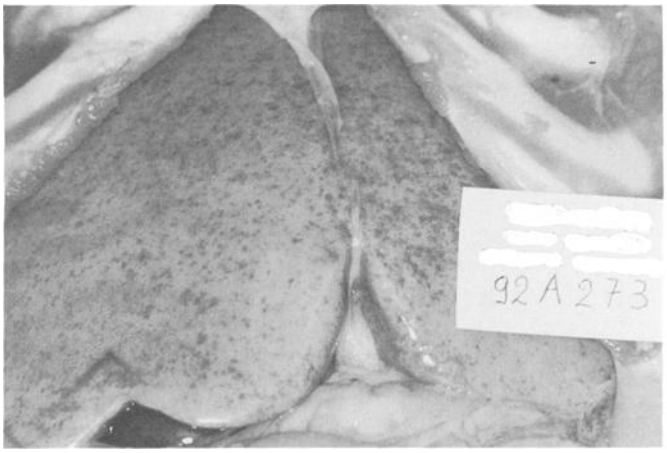


FIG. 1—*Liver: diffuse subcapsular hemorrhaging.*

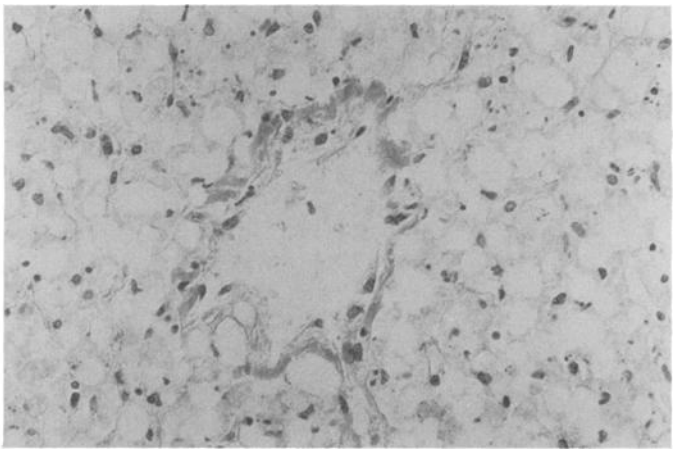


FIG. 2—*Liver: typical massive centrilobular necrosis (Van Gieson's Technique ×200).*

electrolyte imbalance and altered renal and liver function. The first three victims only survived 36–48 h, dying in a toxic coma: the fourth victim survived for five days.

The fifth case, which involved a six-year-old, was atypical. The mushrooms were picked by the girl's grandfather in the woods near their house. The girl ate only one mushroom. Diarrhea and vomiting did not occur until the third day after ingestion of the mushrooms, when there was a sudden worsening of her general condition. On admission to the hospital, the child was in a deep coma with fixed mydriasis, areflexia, absence of response to any stimulus and decerebrate attitude. Blood chemistry showed extremely high ALT (16555 U/L) and AST (10395 U/L), hypoglycemia (3 mg/dL), and 150 s Quick's prothrombin time. Urine sediment contained crystals of cysteine and tyrosine. The patient died a few hours after admission. Following postmortem examination ordered by the hospital authority, death was erroneously attributed to Reye's syndrome (8). Forensic examination was requested and performed in our Department.

All cases were HbSAg negative. The autopsy findings were characterized by yellow complexion due to jaundice, brain edema, subserosal petechiae, lung congestion and signs of hemorrhage, intensely yellow liver of creamy consistency and diffuse subcapsular hemorrhaging (Fig. 1). The kidneys were extremely dark red; there was extravasation of blood especially in the cortical region. The histological examination confirmed stasis in all organs with diffuse hemorrhagic foci. The liver showed typical features of massive centrilobular necrosis, especially in the two cases of longer survival (Fig. 2). In the others, the picture was dominated by vacuolar degeneration of liver cells. In the kidneys, we found acute tubule necrosis (confirmed by biopsy material obtained in the

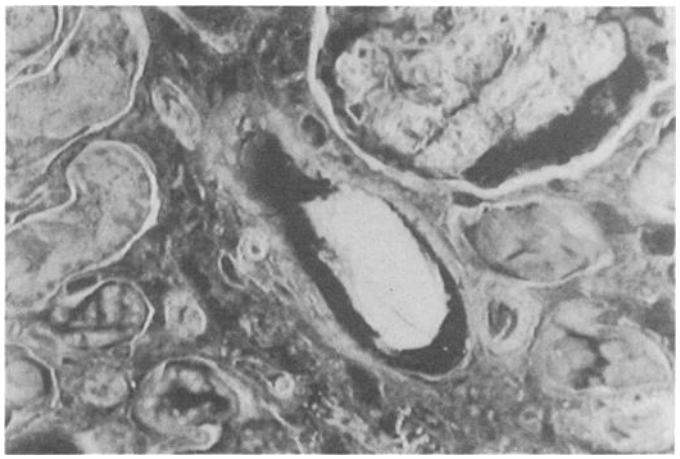


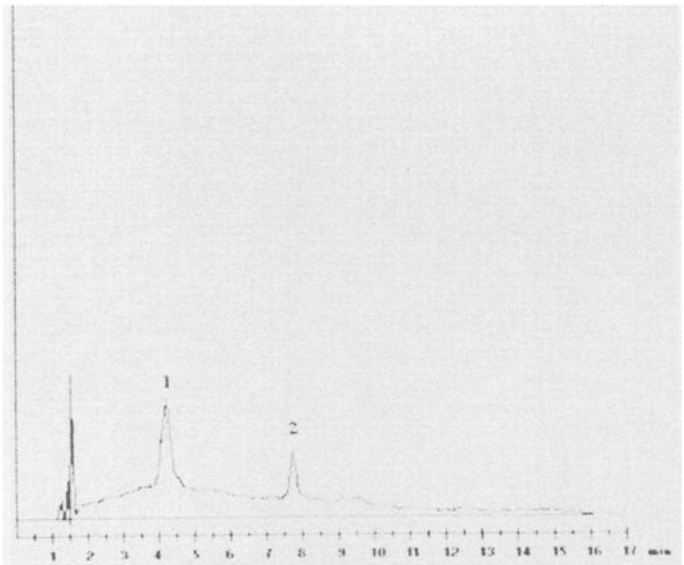
FIG. 3—*Kidney—intratubular cast (Pas modified ×480)*

TABLE 1—*Blood chemistry.*

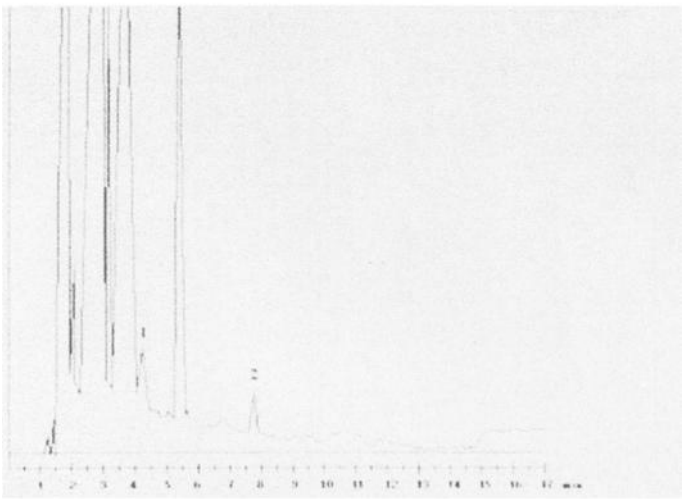
Test	Specimen	Reference Interval	Case 1	Case 2	Case 3	Case 4
Urea nitrogen	Serum	10–20 mg/dL	85	96	91	102
Glucose	Serum	70–105 mg/dL	50	68	60	43
Sodium	Serum	136–146 mEq/L	160	148	150	149
Potassium	Serum	3,5-5,1 mEq/L	3	2,8	2,8	2,5
AST	Serum	10–30 U/L	6500	8700	9600	8230
ALT	Serum	8–20 U/L	7840	7608	8800	7480
Creatinine	Serum	0,6-1,2 mg/dL	6,3	4	6,2	8,6
Bilirubin Total	Serum	0,2-1,0 mg/dL	3,6	6	6,9	7,7
Albumin	Serum	3,5-5,0 g/dL	2,5	2	3	2,5
Fibrinogen	Plasma (Na citrate)	200–400 mg/dL	150	124	180	70
Prothrombin time (Quick method)	whole blood	<20 s	60	75	90	55

hospital) and massive quantities of hyaline casts in the tubules (Fig. 3); in one case there were also many erythrocytes in the tubules.

In case 5, toxicological examination revealed amanitin in the urine, which, together with the morphological data, was sufficient to exclude Reye's syndrome. Amanitin was tested and assayed in biological material by RIA, and later confirmed by HPLC. RIA performed according to the instructions for the amanitin kit produced by Buhlmann Laboratories AG (Postfach, 4123 Allschwil 1, Switzerland) revealed 18 ng/mL of amanitin in the bile. This was confirmed by the finding of α and γ-amanitin in biological material (Fig. 4) when the sample was prepared and analysed according to the method described by Tagliaro (12,13).



HPLC -Chromatogram of γ Amanitin (1) and α Amanitin standards.



HPLC -Chromatogram of bile sample.

(1) - γ Amanitin

(2) - α Amanitin

FIG. 4—*The finding of α and γ-amanitin in biological material.*

Discussion and Conclusions

Our five cases of fatal poisoning by Amanita phalloides confirm the dramatic peculiarity of the syndrome. Forensic diagnosis is based on a protocol for the collection of various kinds of evidence: circumstantial (ingestion of mushrooms), clinical (with the stages of incubation, gastrointestinal symptoms, cytotoxic and comatose), pathological (severe kidney and liver damage and hemorrhagic dyscrasia), histological and toxicological.

Histopathologic examination proved to be important for interpretative, clinical and scientific purposes, reflecting the various stages. Histologic changes in the liver showed three stages. The first stage consisted of hepatocytes sometimes penetrated by erythrocytes and containing vacuoles with weakly eosinophilic granular content; the second stage consisted of lysosomal fusion in the vacuoles giving an acid-phosphatase-positive reaction; the third and final stage consisted of centrilobular necrotic foci and frequent hemorrhagic infiltration of the liver (5,6,9,10) causing a genuine bright yellow atrophy. In the kidneys there was acute tubular necrosis and an enormous number of intratubular casts (1,2,4).

These five, recent and homogeneous cases of a known pathology that has been studied little from a forensic viewpoint, confirm that prospective protocols and methods of interpretation (3,11) must be based on careful histopathological, cytological, chemical and toxicological investigation.

Summary

In the framework of a forensic diagnosis of poisoning by mushrooms, the authors discuss five cases of fatal poisoning due to ingestion of Amanita phalloides, in which careful histological and toxicological investigation was performed. The information thus obtained was extremely useful for an accurate diagnosis.

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