coloration, which is observed in other tissues[22,36], probably depends on the amount of the reaction product within nervous structures. An aminergic innervation by noradrenalin and serotonin is probable in the octopod heart and has been demonstrated in pharmacological experiments[7,11,12,37]. In other molluscan hearts serotonin and dopamine are localized in the nerves by fluorescence microscopy[38] and are therefore possible transmitter substances.

This study demonstrates aminergic and non-aminergic innervation of the cephalopod heart, and as cholinergic effects are also seen in this tissue[11,13], the cephalopod heart may show a remarkable convergence with that of the vertebrates.

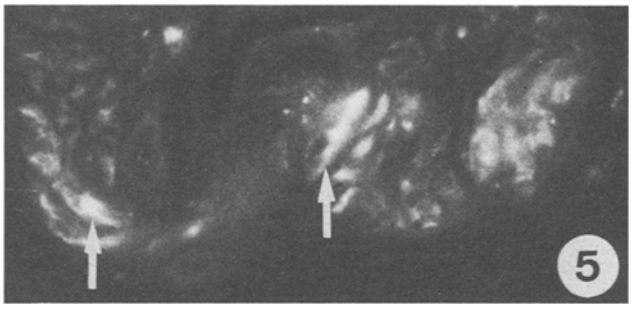

Figure 5. Tangential section through a cardiac nerve trunk. It can be seen that some fibers react negatively, whereas others show strong histofluorescence (arrows). × 2175.

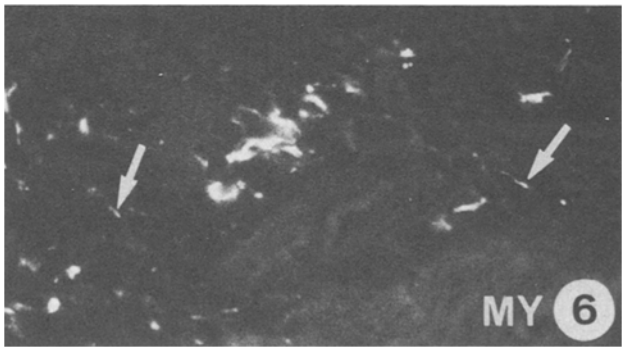

Figure 6. Aminergic fluorescent fibers within the myocardium. Singular varicosities are visible (arrows). × 2175.

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Ethanol diminishes the toxicity of the mushroom *Amanita phalloides*

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Summary. Survival of mice after lethal doses of a lyophilizate from *Amanita phalloides* ('death cap') was markedly increased by single doses of ethanol applied 30 min before or 5 min after the mushroom. Hepatic histopathological damage (confluent necrosis) was largley prevented. Acute, but not chronic, consumption of ethanol may thus influence favorably the outcome of death cap poisoning and should be taken into consideration in the evaluation of therapeutic measures.
Key words. Mushroom poisoning; *Amanita phalloides;* ethanol.

A variety of agents display antagonistic effects against experimental poisoning with extracts of *Amanita phalloides* ('death cap') or against individual toxins of the toadstool such as phalloidin or α-amanitin[1]. Clinically, three treatments have been found to be associated with improved survival, namely penicillin G, silibinin and hyperbaric oxygenation[2]. Since hepatotoxic agents such as carbon tetrachloride protect against the toxicity of phalloidin and the total mushroom extract[1], we also investigated the effects of ethanol. Previous experiments had shown that protracted pretreatment with ethanol did not protect mice against the mushroom extract unless it was combined with small doses of mushroom[3]. We now report that the survival of

mice after lethal doses of a lyophilizate from *Amanita phalloides* is markedly increased by single doses of ethanol applied shortly before or after the mushroom.

Female albino (MAG) mice weighing 20–30 g and fed Nafag pellets and water ad libitum were used. They were injected by the i.p. route with a lyophilizate of *Amanita phalloides* (APL)[4] dissolved in saline and administered in 0.2 ml/10 g b.wt. The LD_{50} of APL was 62.5 mg/kg. Fatalities occurred mostly between 6 and 48 h and never after day 15. Ethanol was diluted with saline and applied i.p. as a 30% solution. 3000 mg/kg ethanol were thus administered in a volume of 0.1 ml/10 g b.wt and 4500 mg/kg in 0.15 ml/10 g. The LD_{50} of ethanol was ~ 8300 mg/kg. Blood ethanol levels after i.p. administration of 4500 mg/kg ethanol to normal mice were 4.9 mg/ml after 15 min, 4.8 mg/ml after 30 min, 4.1 mg/ml after 2 h, 1.4 mg/ml after 4 h and not measurable after 8 h. When ethanol was given 5 min after the APL, ethanol concentrations were 6.0, 5.7, 4.4 and 2.1 mg/ml at 15 min, 30 min, 2 h and 4 h, respectively. With ethanol administration 30 min before the APL, ethanol concentration 1 h and 2 h after its injection was not significantly changed, but increased to 2.4 mg/ml at 4 h.

Table 1 shows that in mice treated at 5 min post-poisoning with 4500 mg/kg ethanol, survival after 125 mg/kg APL ($2 \times LD_{50}$) was increased from 0% to 58%, after 100 mg/kg APL from 7% to 83% and after 75 mg/kg APL from 17% to 100%. Treatment with 4500 mg/kg ethanol at −30 min or with 3000 mg/kg at either −30 or +5 min was only slightly less effective. 2000 or 6000 mg/kg of ethanol afforded inferior but still significant increases in survival, but not 1000 mg/kg ethanol or the administration of otherwise effective doses at +30 min.

The histological analysis of the livers of the APL-poisoned mice revealed striking differences (table 2). Ethanol prevented or reduced confluent necrosis (CN) in the groups poisoned with 50, 70, 82.5 and 100 mg/kg APL. CN in ethanol-treated mice, if present at all, was observed in later stages as compared to animals with the same doses of APL but without ethanol. In both ethanol-treated and non ethanol-treated mice surviving CN, necrosis was cleared by scavenger cells and cell loss was compensated by regeneration, as evidenced by mitosis. In the ethanol-treated groups, however, mitosis sets in with delay, e.g. with 50 mg/kg APL after 120 h as compared to 48 h in mice not treated with ethanol.

Table 1. Survival of mice poisoned with a lyophilizate from *Amanita phalloides* (APL) and treated with single doses of ethanol

APL (mg/kg)	Ethanol (mg/kg)	Applied at (min)	Survival at 15 days	Survival (%)
75	—	—	5/30	17
75	3000	−30	5/6	83*
75	4500	−30	11/12	92*
75	3000	+5	6/6	100*
75	4500	+5	23/23	100*
100	—	—	2/30	7
100	3000	−30	14/24	58*
100	4500	−30	14/18	78*
100	3000	+5	11/18	61*
100	4500	+5	10/12	83*
125	—	—	0/18	0
125	3000	−30	5/18	28**
125	4500	−30	6/12	50*
125	3000	+5	8/18	44*
125	4500	+5	7/12	58*

Mice were poisoned with APL applied by the i.p. route. Ethanol was injected i.p. either 30 min before (−30) or 5 min after (+5) APL. Controls received 0.15 ml of saline per 10 g b.wt. Survival was followed up to day 15. Statistical significance (p) according to the chi-square test. Survival: No. of surviving mice/total No. of mice. *$p < 0.001$; **$p < 0.02$.

Experiments designed to investigate which category of toxins from *Amanita phalloides* are antagonized by ethanol were not conclusive. Neither the toxicity of phalloidin nor that of α-amanitin were influenced by ethanol (table 3). It may be that ethanol affects only the combined actions of phallotoxins and amatoxins.

The complexity of the interaction of ethanol with hepatotoxic agents is illustrated by the opposite effects of ethanol on the hepatotoxicity of acetaminophen and carbon tetrachloride, which are both subject to biotransformation by hepatic microsomal enzymes to reactive metabolites[5]. The toxicity of acetaminophen is reduced by acute ethanol through inhibition of its biotransformation by mixed-function oxidation[6,7], whereas chronic ethanol increases the hepatotoxicity of acetaminophen in association with enhanced production of reactive metabolites[8]. On the other hand, carbon tetrachloride-induced hepatotoxicity, which also fits the 'toxic metabolite hypothesis', is increased not only after withdrawal from chronic ethanol consumption[9] associated with increased activity of a variety of

Table 2. Histopathological changes in livers of mice poisoned with APL and treated or not treated with ethanol

APL (mg/kg)	Ethanol	Hours after APL	Confluent necrosis (% lobular area)	Mitoses[d]
50	−	8	90, 55[a]	0, 0
50	+	8	0, 0	0, 0
50	−	24	50, 0[b]	0, 0
50	+	24	5, 15	0, 0
50	−	48	0[b], 15	+++, ++
50	+	48	0, 0	0, 0
50	−	120	0, 0	0, +
50	+	120	0, 0	++, +
82.5	−	8	90, 90, 95	0, 0, 0
82.5	+	8	0, 0, 0	0, 0, 0
82.5	−	24	75	0
82.5	+	24	0, 0, 0	0, 0, 0
82.5	−	48	0, 15[c]	+++, ++
82.5	+	48	25[c], 0, 0	+++, 0, 0

[a] Figures correspond to individual mice. [b] Macrovesicular degenerative changes. [c] In stage of restoration. [d] Frequency of mitosis graduated from 0 to +++. Groups of mice were poisoned with APL. After 5 min, the animals were given either 4500 mg/kg ethanol (+) or a similar volume of saline (−) i.p. After 8, 24, 48 and 120 h, animals were killed and their livers removed, fixed in 3% buffered formalin and processed for histological examination by conventional techniques. Estimation of the degree of confluent necrosis was done by a semiquantitative assessement. An average normal mouse liver lobule is made up of liver cell plates connecting central veins (hepatic venules) with the periportal limiting plate, each plate consisting of 12 rows of liver cells. Confluent necrosis was of the 'pericentral type', starting in the perivenular region (zone III of Rappaport) and extending towards the periportal region (zone I of Rappaport). By counting the remaining intact rows of liver cells, the number of rows which had undergone necrosis could be estimated and were expressed as percent of lobular area (with 12 rows taken as 100%).

Table 3. Effect of 4500 mg/kg ethanol on survival of mice from poisoning with the death cap toxins phalloidin or α-amanitin

Toxin	Dose (mg/kg i.p.)	Ethanol (mg/kg i.p.)	Applied at (min)	Survival at 15 days	Survival (%)
Phalloidin	6	—	—	0/9	0
Phalloidin	6	4500	−30	1/7	14
Phalloidin	6	4500	+5	1/10	10
α-Amanitin	1	—	—	5/23	22
α-Amanitin	1	4500	−30	3/23	13
α-Amanitin	1	4500	+5	6/23	26

Phalloidin and α-amanitin (courtesy Prof. H. Faulstich, Heidelberg) were dissolved in saline and injected by the i.p. route in 0.2 ml/10 g b.wt. The controls received only saline. The differences in survival after the i.p. injection of ethanol were not statistically significant.

drug metabolizing enzymes[10], but also with acute ethanol administration[11], which is known to inhibit microsomal drug metabolism[10,12]. Since no metabolic changes have been demonstrated for Amanita toxins, it is unlikely that these considerations apply to the mechanism by which ethanol affords protection against death cap toxicity. It is also not possible to conclude from a study assessing in mice the influence of ethanol given as an oral dose of 4800 mg/kg 16 h earlier on a variety of hepatotoxic agents including carbon tetrachloride and α-amanitin[13], that the observed prevention of the rise of serum enzymes due to α-aminitin was caused by a metabolic interference involving microsomal enzymes.

As chronic application of ethanol with a last dose at 32 or 56 h before the APL did not reduce its toxicity[3], the protection seems to be due to an acute effect of ethanol on the APL-induced liver damage. One possibility is that ethanol interferes with the uptake of the mushroom toxins into liver cells, either by blocking receptors or a transport system[14], or by causing structural disorders of membrane lipids[15,16]. The fact that the toxins are normally taken up by liver cells within 15 min would explain why ethanol given 30 min after APL did not ameliorate survival. Finally, acute ethanol administration may affect the disturbances in cellular calcium homeostasis caused by the toxins[17].

According to our finding, simultaneous ethanol interferes with the toxicity of the toadstool. This observation could contribute to explaining why the mortality of death cap intoxication in children below 10 years of age is much higher (51.3%) than in adults (16.5%)[2], although the dose/b.wt relation has to be considered. So far, a) age and b) latency period between the meal and appearance of the first symptoms have been ascertained as prognostic variables in death cap poisoning[2]. Although blood ethanol levels in ordinary meals comprising mushrooms are likely to be lower than those observed in our experiments, the present observation clearly suggests that the ingestion of alcoholic beverages with the meal should also be taken into account when therapeutic measures in clinical *Amanita phalloides* intoxication are evaluated.

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Forskolin supresses seizures induced by pentylenetetrazol in mice

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Summary. Forskolin, an adenylate cyclase activator when injected s.c. (1 mg/kg) in mice, caused an elevation of cAMP in the forebrain and cerebellum of up to 170% and 130%, respectively. The treatment was found to prevent seizures induced by pentylenetetrazol. This suppression had subsided 30 min after the injection, when cAMP level was again normal in the cerebellum but still elevated in the forebrain.

Key words. Mouse brain; convulsions; forskolin; anti-convulsant drugs; cAMP, cerebral.

Forskolin, a diterpene, is isolated from a plant (*Coleus forskohlii*[1]). The *Coleus* sp. have been described in ancient medical texts as having medicinal properties against various diseases including central nervous system diseases like insomnia and convulsions[2]. Recently, forskolin was shown to be a highly potent and specific activator of various mammalian adenylate cyclase systems in intact cells, membranes and detergent-dispersed membranes[3–5]. Forskolin can mimic biological actions of hormones through an increase of cAMP content[6,7]. It has been demonstrated that experimental seizures alter the cyclic nucleotide level in the brain; electroconvulsive shock[8,9] and several convulsant drugs[10,11] can elevate the level of cAMP after the onset of the seizures in the mouse brain. Ferendelli hypothesized that this elevation may be involved in mechanisms inhibiting the seizure activity since he found that animals pretreated with cAMP-lowering agents (reserpine, propranolol or aminophylline) showed an enhanced sensitivity to seizures induced by pentylenetetrazol, while cAMP or its derivatives depress the electrical activity of neurons[12,13].

In the present investigation, we wanted to see whether forskolin is able to affect seizures induced by the convulsant drug, pentylenetetrazol.

Materials and methods. Experiments were performed on male ICR mice weighing 35 to 37 g, 2 months old, which were maintained on a 12:12 h light-dark cycle. Forskolin, purchased from Calbiochem-Behring Corp., was disolved in ethanol and diluted with Ringer solution to 8.3% ethanol (V/V) just before its use, and was injected s.c. in 0.1 to 0.125 ml. Pentylenetetra-