agents, such as uranyl nitrate[8] or potassium dichromate[9], specifically enhanced the *N*-methylnicotinamide accumulation in renal cortical slices.

In our experiments, after repeated THAM administrations to rats of different ages, an enhancement of THAM accumulation was observed, except in newborns (Figure 1). This stimulatory effect can be interpreted as a specific substrate stimulation of the organic base transport system. Furthermore, there is an accordance with the results obtained in vivo[2,3].

In principle, a $Q_{S/M} > 1$ measured in renal cortical slices is an index of the ability of the proximal tubular cells to maintain a concentration gradient. However, the physico-chemical properties of the foreign compounds, especially the lipid solubility, the dissociation rate, and the binding rate for renal tissue proteins must be considered. Under steady-state conditions, the accumulation process is the sum of the influx into the tubular cells, a possible intracellular retention, and the efflux from cells back into the incubation medium. THAM influx is the result of a carrier-mediated component as well as an energy-independent component (Figure 2). Passive THAM uptake can take place by diffusion and possibly by non-specific protein-binding to renal tissue proteins. In the incubation medium (pH 7.4), about 30% of THAM (pKa 7.8)[10] is not dissociated, and therefore freely diffusible in this case. In contrast to the THAM accumulation, the PAH accumulation is produced by the carrier for organic acids alone. There is no passive PAH diffusion into the tubular cells, because PAH (pKa 3.8)[11] is completely dissociated in the incubation medium. Furthermore, there is no protein binding for PAH. A passive diffusion of *N*-methylnicotinamide into the tubular cells cannot be stated either[9], whereas a tissue binding was suggested previously[12].

Stimulation of THAM transport by the organic base transport system may involve changes of the carrier protein synthesis[13], protein binding sites[9], or membrane permeability.

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RNA Synthesis in α-Amanitin-Poisoned Rats: Prevention of Recovery by Inhibition of Protein Synthesis

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Summary. The treatment with cycloheximide of rats previously poisoned with α-amanitin hinders the recovery of RNA synthesis observed in the liver of rats treated with α-amanitin alone. The recovery of RNA synthesis can be ascribed to the capability of poisoned rats to synthesize new RNA-polymerase II.

α-amanitin, the main toxin in the toadstool *Amanita phalloides*[2], exerts its cytopathic effect by the inhibition of RNA-polymerase II[3–5]. The toxin causes liver and kidney necrosis in the mouse, but not in the rat, in which it produces reversible lesions only in the liver[6–8]. This fact was explained by the incapacity of the epithelial cells in rat kidney tubules to readsorb amanitin from preurine[7], so that the different toxicity of amanitin for mice and rats was related to the different time for which the toxin remains in the organism. This hypothesis was not supported, however, by the recent finding that α-amanitin disappeared from the blood of poisoned mice as early as 4 h after toxin injection[9]. Thus we can propose that the different effect of α-amanitin in rats may be due either to 1. a fast dissociation of the RNA polymerase-α-amanitin complex in rats, or to 2. a capability of rats to synthesize new RNA-polymerase II. To ascertain which of the two hypotheses is the right one, we have studied the synthesis of RNA in the rat liver after α-amanitin poisining, in an attempt to detect the recovery time of the RNA synthesis. At this time, that is when the RNA synthesis begins to increase, we have injected the rats with cycloheximide, an inhibitor of protein synthesis[10,11].

Experimental. Young male rats (body weight 100–110 g) of Wistar strain were divided into 8 groups of at least 3 animals: 6 groups were injected i.p. with α-amanitin (50 μg/100 g body weight) and killed 6, 7, 10 and 12 h thereafter; 2 groups of these also received cycloheximide (0.15 mg/100 g body weight) at either 6 or 7 h, and were killed at 10 and 12 h respectively. Of the remaining 2 groups, one received only cycloheximide at the same dosage and was killed 4 h thereafter; the last group of 7 rats received saline and were used as controls.

RNA synthesis was measured by the rate of incorporation of 6-^{14}C orotic acid (57 Ci/ml, Radiochemical Center Amersham) according to MUNRO and FLECK[12]. Rats were killed 10 min after i.p. injection of orotic acid (3 μCi/100 g body weight). For the electron microscopy, liver samples were processed as reported by DERENZINI and BONETTI[13].

Results and discussion. As shown in Figure 1, the synthesis of RNA, strongly inhibited at 6 h, was markedly increased 10 and 12 h after α-amanitin treatment (at the latter time, the value of RNA synthesis overcome the control one). Concurrently we observed clear changes in the chromatin pattern: in fact, the chromatin, strongly condensed 6 h after poisoning (Figure 2), appeared almost completely decondensed 12 h after the toxin injection (Figure 3). Therefore, the phenomena related to the recovery of RNA synthesis had to be circumscribed between the 6th and the 12th h. For this reason, 6 and 7 h after α-amanitin injection, we further injected the rats with cycloheximide. At the dose used, cycloheximide reduced protein synthesis in the rat liver to about 5% of the control level, without affecting the synthesis of RNA[14]. In our experiments, we observed no modifications

of the synthesis of RNA 4 h after injection of cycloheximide alone. On the contrary, when cycloheximide was injected 6 or 7 h after α-amanitin poisoning, the synthesis of RNA measured 4 and 5 h later (and 10–12 h after α-amanitin injection) appeared to be strongly reduced, i.e. the recovery of the synthesis of RNA observed 10 and 12 h after the α-amanitin injection alone was clearly prevented (Figure 1). Correspondingly, the chromatin decondensation, so evident in rat hepatocytes 12 h after α-amanitin treatment and indicative of an enhancement of the synthesis of RNA[15], was hindered; moreover the ultrastructural pattern of chromatin in the hepatocytes, 12 h after α-amanitin + cycloheximide treatment, was very similar to that visible 6 h after the injection of α-amanitin alone (Figure 4).

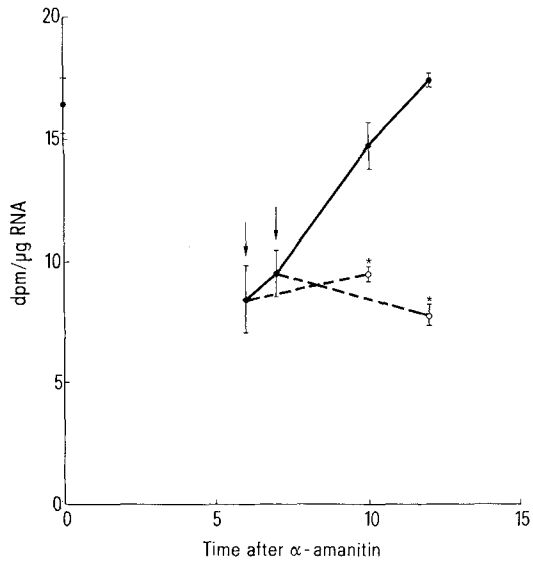

Fig. 1. RNA synthesis in hepatocytes of rats treated with α-amanitin alone (.———.) or α-amanitin plus cycloheximide (.– – – –.). Rats received α-amanitin at 0 time and were killed as indicated in the Figure. Arrows indicate the time of i.p. injection of cycloheximide after α-amanitin treatment in 2 groups of rats. Results are expressed as means ± SEM. For statistical evaluation of results, Fisher's test was used and a difference between 2 mean values was regarded as significant (*) if p was no greater than 0.05. Fisher's p are calculated between mean values of the α-amanitin plus cycloheximide group and the correspondent α-amanitin treated one. Results are expressed as dpm/µg RNA and have been corrected for the acid-soluble radioactivity (dpm/mg tissue) measured in the supernatant, after the first acid precipitation in the method of MUNRO and FLECK[12].

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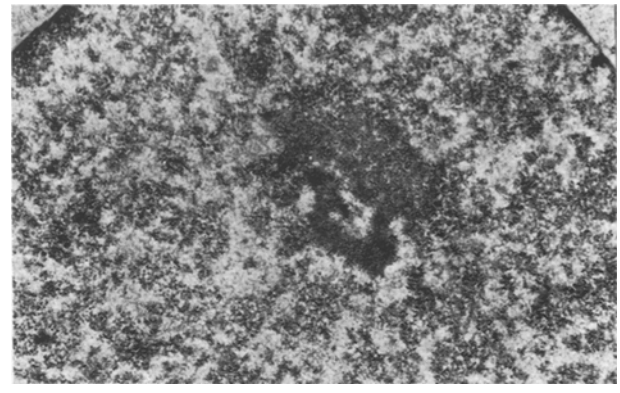

Fig. 3. Hepatocyte nucleus in rat 12 h after α-amanitin treatment. Almost all the chromatin is in the decondensed form and it spreads to every part of the nucleoplasm. The nucleolar material has assumed the pattern of the rebuilding nucleolus. Aldehyde fixation. ×26,000.

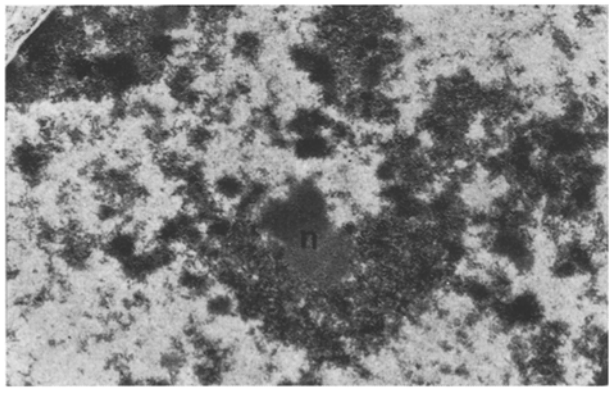

Fig. 2. Hepatocyte nucleus in rat 6 h after α-amanitin treatment. The chromatin is strongly condensed, the nucleolus is broken up into many fragments, in one of which (n) granules and fibrils are clearly segregated. Aldehyde fixation. ×26,000.

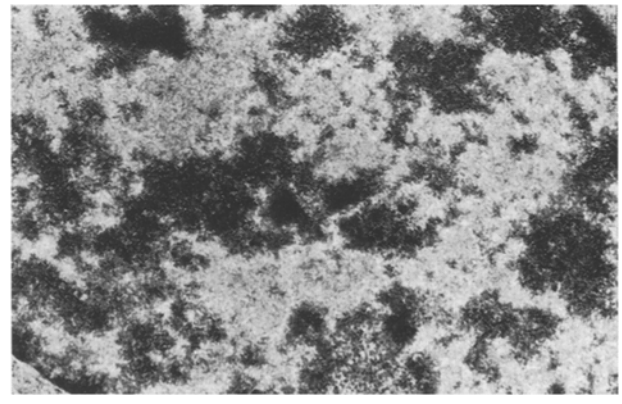

Fig. 4. Hepatocyte nucleus in rat after double treatment with α-amanitin (7 h) and cycloheximide (5 h). The chromatin is still strongly condensed as in Figure 2. Aldehyde fixation. ×26,000.

Therefore, our results have shown that, in the rat liver 1. the synthesis of RNA was strongly reduced by α-amanitin until 6 h after poisoning; 2. there was a complete recovery of the synthesis of RNA between the 6th and the 10–12th h after α-amanitin treatment; 3. this recovery was hindered by the inhibition of protein synthesis with cycloheximide. Thus we can exclude the possibility that in the rat liver the recovery of the synthesis of RNA may be due to a dissociation of the α-amanitin-RNA polymerase complex. Our results fit reasonably well with the hypothesis that in the rat liver the recovery of the synthesis of RNA may be due to the synthesis of new molecules of RNA polymerase II.

The capability of the rat liver to synthesize the RNA polymerase II may be due to a long life of the mRNA for this enzyme. α-amanitin, by lowering the synthesis of extranucleolar RNA, induces also an inhibition of the mRNA synthesis[16]. Therefore, if the lifetime of the mRNA molecules is longer than the clearance time of α-amanitin from the organism, new enzyme molecules would not be inhibited by the toxin.

In conclusion, the difference in sensitivity between rats and mice to α-amanitin may be explained on this basis: liver RNA synthesis is inhibited by the toxin both in rats and in mice, but a longer life of mRNA in the rat prevents the inhibition of protein synthesis. This is in agreement with the finding that in mouse liver the protein synthesis, 18 h after α-amanitin poisoning, was reduced to the 50% level of the controls, whereas in rat liver it was practically unaffected. Moreover, this inhibition of protein synthesis was found to be due to a lack of mRNA[17].

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Effect of Brocresine on Conditioned Avoidance Behavior in Mice[1]

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Summary. Brocresine, an inhibitor of brain histamine biosynthesis, has been found to impair the ability of mice to avoid shock in a shuttle box CAR test; escape performance was unaffected in these studies.

An increasing body of evidence has appeared in recent years supporting the concept that histamine is a neurotransmitter in the central nervous system. Histamine is nonuniformly distributed in the brains of mammals and is synthesized in nerve endings, stored in synaptic vesicles and its release is enhanced by potassium-induced depolarization. The turnover rate of brain histamine has been estimated to be less than 1 h and this rate is reduced after barbiturate administration[2–4]. While the functional role of histamine is obscure at this time, its central administration has been reported to modify such vegetative functions as thermoregulation[5], water intake[6], water balance regulation[7] and the emetic response[8], as well as alter continuous (Sidman) avoidance[6] and self-stimulation[9] behaviors. This study was designed to determine whether brocresine-induced inhibition of histamine biosynthesis could modify the acquisition, retention and performance of a learned behavior (conditioned avoidance responding) in mice. Brocresine, an inhibitor of histidine decarboxylase[10,11], is capable of reducing rodent brain histamine levels by 40–50%[12].

Methods. Male albino CD-1 mice (18–25 g) were employed in these experiments in a typical shuttle box[13]. Animals were trained to perform a shuttle box conditioned avoidance response (CAR) as follows: the mouse was placed at one end of the box and, after a 5-sec environmental exposure period, 5 sec of buzzer was presented, followed by 15 sec of footshock (60 cycle alternating current; 2 milliamperes delivered through a shock scrambler) in the continued presence of buzzer. To avoid or escape a shock, the mouse was required to reach a 'safe area' platform placed at grid level at the opposite end of the shuttle box. Each mouse was given 10 trials in the morning and afternoon until the animal was able to avoid shock in 9 out of 10 consecutive trials. After achieving this 90% avoidance criterion, the test animal was used as its own control receiving saline and brocresine in the morning and afternoon, respectively, 30 min prior to testing. In experiments designed to evaluate drug effects on the acquisition of CAR, behaviorally naive mice were given up to 100 consecutive trials or until each animal achieved the 90% avoidance criterion.

Results and discussion. At doses up to 300 mg/kg, i.p. administered 5–120 min prior to testing, brocresine failed to induce minimal neurotoxicity or muscle incoordination when evaluated by the inability of mice to remain on a horizontal rod rotating at 6 rpm for 1 min. Greatest depression of spontaneous motor activity was observed 30–60 min after drug administration.

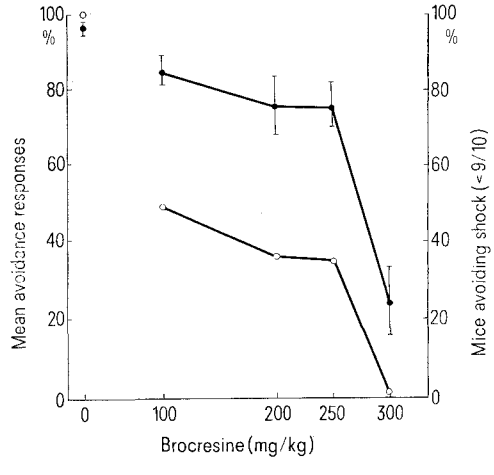

Fig. 1. Effect of brocresine and saline on avoidance responding in CAR trained mice. ●, mean percent avoidance responses ± SEM out of a total of 80 trials at each dose of brocresine; each of 8 animals was used as its own control. ○, percent of 8 mice avoiding shock less than 9 times in 10 trials. All doses of brocresine significantly ($p < 0.05$) impaired performance.