Discussion. Exogenously administered histamine is normally metabolized by two routes: firstly by deamination with diamine oxidase to form imidazole acetic acid[5,6] and then conjugation to form ribosylimidazole acetic acid[7,8], and secondly by methylation[9] and then deamination (by monoamine oxidase) to give methylhistamine and methylimidazole acetic acid, respectively[10,11]. The relative importance of these two routes varies in different species with deamination and riboside conjugation predominating in rat and guinea pig[10,11] and methylation in man[12], cat and dog[10,11].

Our studies confirm that deamination and ribosylation is the principal pathway of histamine metabolism in rat[9,11] and is of lesser importance in mouse[10] and man[12]. The studies show in addition that imidazole acetic acid is almost completely converted to the ribosyl conjugate in rat and partially so in mouse. The conjugation is inhibited reversibly by aspirin and sodium salicylate in all three species studied. Inhibition of the enzyme imidazoleacetate phosphoribosyl transferase[13,14] is probably the mechanism for the reduction of conjugation *in vivo*[2]. Although the levels of salicylates required to inhibit the enzyme *in vitro* are 3–5 times lower than those required to inhibit conjugation *in vivo*[2], unequal distribution or fluctuating levels in tissues may account for this difference. The inhibition is specific in that glucuronide and ethereal sulfate conjugation of estrone and morphine are not blocked by aspirin[1].

The salicylates have an extraordinary wide spectrum of biochemical activities, some of which have been proposed as being responsible for the pharmacological activities of these drugs (for the older literature, see ref.[15]). Of current interest is the ability of salicylates to inhibit prostaglandin synthesis in a variety of tissues and preparations[16]. Since all anti-inflammatory drugs share this property, and the order of potency in inhibiting prostaglandin synthesis correlates well with the anti-inflammatory activity of these drugs, it is felt that inhibition of prostaglandin synthesis contributes to anti-inflammatory actions of these drugs. Whether the inhibition of ribosyl conjugation is of pharmacological significance requires further study. Some preliminary comments can, however, be made.

The inhibition of ribosyl conjugation does not appear to impair the ability of the body to destroy histamine, and for this reason we do not feel that aspirin influences inflammation through its effect on histamine metabolism. The effect on L-histidine metabolism may be quantitatively more important, since this amino acid is the principal source of imidazole acetic acid in the body[17]. Although it is usually assumed that imidazole acetic acid is pharmacologically inactive (for example, see ref.[18]) this acid does have pronounced central effects[19]. It is a potent inhibitor of cat cortical neurone activity[20]. In doses of 100 to 400 mg/kg i.p. analgesia and with the higher doses narcosis have been observed in mice[3,4]. It was also reported that imidazole acetic acid had similar effects in rats and guinea-pigs[3]. Experiments in this laboratory (unpublished) have shown that with these doses narcosis and analgesia are produced in mouse but not in rat. It is of interest that mouse, unlike rat, does not completely conjugate imidazole acetic acid and is therefore less capable of inactivating the acid. If inhibition of imidazole acetic acid metabolism contributes to the analgesic action of salicylates, other mechanisms must operate for non-salicylate anti-inflammatory drugs, since these drugs do not block the metabolism of this acid.

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Enhancement of Tubular Organic Base Accumulation in Renal Cortical Slices by Repeated Administrations of *Tris*-Hydroxymethyl Aminomethane, Tromethamol (THAM) to Rats of Different Ages

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Summary. After repeated THAM administrations to rats of different ages, an enhancement of THAM accumulation in renal cortical slices was observed, except in newborns. This effect can be interpreted as a specific substrate stimulation of the organic base transport system.

After repeated THAM administrations, the renal excretion of this organic base was accelerated in rats of different ages, except in newborns[2]. This effect was not caused by a decrease in tubular reabsorption rate[3]. Enhancement of THAM accumulation in renal cortical slices from THAM pretreated rats could prove the specific substrate stimulation of the organic base transport system, because glomerular filtration or renal blood flow cannot influence the result. The objective of the present study was to investigate whether tubular THAM accumulation in vitro can be increased by repeated THAM administrations to rats of different ages. In addition, the

oxygen-requiring component of THAM accumulation was determined by inhibiting energy supply by using 2,4-dinitrophenol (DNP) or nitrogen atmosphere.

Material and methods. Wistar rats (Jena) of our colony breed were used. Younger rats of both sexes and female adult animals were i.p. administered 94 mg THAM/100 g b.wt. twice daily for 4 days, while controls received saline. 16 h after the last administration, renal cortical slices from 5-, 15- and 55-day-old rats were prepared as previously described[4]. Kidney slices were incubated in Krebs-Ringer phosphate buffer (pH 7.4), which contained 5.5×10^{-4} *M* THAM. All incubations were carried out in a Warburg apparatus at 25 °C under a gas phase of 100% oxygen for 120 min[4,5]. In order to estimate the carrier-mediated transport component, incubation was carried out under nitrogen atmosphere or in the presence of 1.0×10^{-4} *M* DNP.

THAM was determined by the method of ROSEN[6]. THAM accumulation in renal cortical slices from control animals and THAM pretreated rats was compared. Accumulation is expressed as slice to medium ratio ($Q_{S/M}$). Arithmetic means ± standard error are given. Differences between means were statistically analyzed using student's *t*-test.

Results. THAM accumulation is different in renal cortical slices from newborn, young and adult rats (Figure 1). Repeated THAM administrations to rats of different ages produce an enhancement of THAM accumulation, except in newborns. The stimulation is most distinct in adults.

Figure 2 demonstrates the effects of anaerobic incubation or uncoupling of oxidative phosphorylation on THAM accumulation in comparison with PAH accumulation. Under both conditions, THAM accumulation is reduced to an age-dependent extent. Likewise, the diminution is most distinct in renal cortical slices from adult rats. In contrast to THAM accumulation, the PAH accumulation is absolutely inhibited in all age groups; slice to medium ratios have the value of 1.

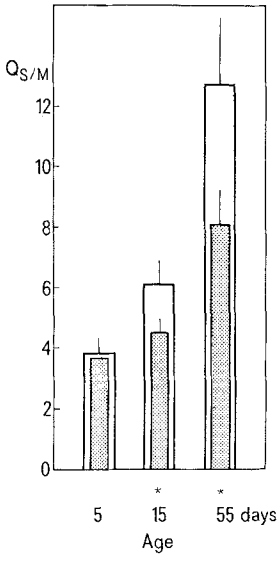

Fig. 1. Effects of repeated THAM administrations in vivo on THAM accumulation in renal cortical slices from rats of different ages. Experiments were performed as described in methods. Columns indicate means (± SE) from 6–12 samples. ▒, Controls; □, THAM pretreatment. Asterisks indicate values significantly different from respective controls ($p \leq 0.05$).

In the Table, the energy-independent THAM uptake in renal cortical slices from control animals and THAM-pretreated rats is compared. Passive uptake of THAM is different depending on age. THAM pretreatment enhanced the passive uptake in slices from 15- and 55-day-old rats, but not significantly.

Discussion. An age-dependent development of the renal organic base transport system, measured by N-methylnicotinamide accumulation in renal cortical slices, has already been documented[7]. Treatment of young rats with organic base substrates, such as *N*-methylnicotinamide or tetraethylammonium, did not result in stimulatory effects, whereas treatment of rats with nephrotoxic

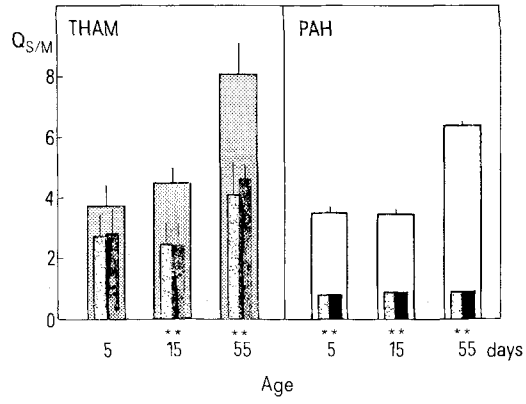

Fig. 2. Effects of N_2 atmosphere or DNP on the THAM accumulation in comparison with their effects on the PAH accumulation measured in previous studies[5]. Bars present mean values (± SE) obtained after 120 min of incubation. At the beginning 5.5×10^{-4} THAM or 8.5×10^{-5} *M* PAH were added to the incubation medium. Both concentrations were most suitable under our experimental conditions. Experiments were performed as described in methods (n = 6–12). ▒, THAM controls; □, PAH controls; ▨, N_2 atmosphere; ■, DNP. Asterisks indicate those values significantly different from their respective controls ($p \leq 0.05$).

Energy-independent component of THAM uptake in renal cortical slices from control animals and rats pretreated with THAM

Age of animals (days)	Passive uptake of THAM ($Q_{S/M}$) Nitrogen atmosphere		2,4-Dinitrophenol	
	Control	Pretreatment	Control	Pretreatment
5	2.6 ± 0.8	2.7 ± 0.7	2.7 ± 0.9	2.4 ± 1.0
15	2.5 ± 0.7	3.3 ± 0.5	2.4 ± 0.9	3.5 ± 0.6
55	4.1 ± 1.1	6.1 ± 0.9	4.6 ± 0.5	5.2 ± 0.9

Active transport component was blocked by DNP or N_2 atmosphere. Experiments were performed as described in methods. Each value represents the mean (± SE) from 4–8 samples.

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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