TMA also appears to inhibit the development of physical dependence, as indicated by naloxone-precipitated jumping (Table III), i.e. more naloxone is required to produce 50% jumping in TMA-treated dependent mice (potency ratio 1.54). Control naive mice did not jump with doses of naloxone up to 100 mg/kg, but TMA-treated naive mice jumped with 75 mg/kg naloxone (40%) and 100 mg/kg (20%). Below 75 mg/kg and above 100 mg/kg, jumping was not observed. The reasons for the apparent sensitization to large doses of naloxone in naive mice treated with TMA are not clear, but this incidental finding does not invalidate the results in Table III which were obtained with much lower doses of naloxone (0.5–5.0 mg/kg).

Discussion. TMA, like cycloheximide, can inhibit the development of morphine tolerance and physical dependence. Comparisons in our laboratory indicate that TMA is about as potent, weight for weight, as cycloheximide in preventing tolerance, but only about 20% as effective in inhibiting physical dependence. It can be tentatively concluded from these results that morphine tolerance and physical dependence both have underlying mechanisms which depend in some way on the formation of Hm somewhere in the brain. However, TMA in the dose used in these experiments (100 mg/kg) did not in fact significantly lower whole brain Hm levels 72 h after administration (Table II). This is in conflict with the findings of MENON et al.[10] in rats, and may be due to species difference. The absence of significant reduction in whole brain Hm after 100 mg/kg TMA does not, of course, rule out localized depletion in areas related more specifically to the central actions of morphine. Furthermore, the measurements were made at one point only in time (72 h after TMA injection), and ideally brain histamine levels should be determined at other time intervals. Higher doses of TMA, e.g. 200 mg/kg, could not be used to increase the chance of obtaining measurable histamine depletion because of substantial mortality (40%). MENON et al.[10] report an LD_{50} of 350 mg/kg i.p. for their strain of mice.

Effect of Substrate Pretreatment on Renal Organic Ion Transport in the Adult Rat[1]

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Summary. The ability of renal cortical slices to accumulate PAH and NMN was not significantly affected by pretreatment of adult rats with large doses of PAH. Pretreatment of adult rats with THAM significantly increased PAH accumulation but had no effect on NMN. Inulin and PAH clearance and filtration fraction were significantly decreased by PAH pretreatment but unaffected by THAM pretreatment. The effects of pretreatment on transport are probably due to non-specific toxicity.

Renal organic anion transport capacity is less in the newborn that in the adult[4–6]. A major stimulus to development of transport is substrate availability[7,8]. Exposure of newborn animals to increased substrate load alters the rate of transport development. HIRSCH and HOOK[6,9,10] observed that *p*-aminohippuric acid (PAH) accumulation into renal cortical slices from neonatal rats and rabbits was significantly increased by pretreatment with penicillin. There was, however, no effect on transport capacity in adult animals[9]. It was concluded that a finite number of transport sites existed in the kidney and that after full development further stimulation could not be produced[9]. BRÄUNLICH et al.[11] and BERNHARD et al.[12] recently reported that pretreatment of adult rats with large doses of PAH or *tris*-hydroxymethylaminomethane (THAM) enhanced the urinary excretion of PAH and THAM, respectively. The purpose of this study was to specifically determine the effect of PAH and THAM pretreatment on renal transport of organic ions. Transport of PAH and the organic base *N*-methylnicotinamide (NMN) was quantified in vitro at steady state using renal cortical slices. Transport of PAH was also quantified in vivo in clearance experiments.

Methods. Adult, male Sprague Dawley rats approximately 50 days of age were purchased. On day 55 treatment was begun. One group of animals received 300 mg PAH/100 g body weight. The second group received 94 mg THAM/100 g body weight. Both compounds were administered i.p. in a total volume of 5 ml. Controls received saline. All solutions were adjusted to pH 7.4 immediately prior to injection. Animals were treated twice daily for 4 days. Transport was measured on the 5th day.

Organic ion transport capacity was determined using renal cortical slices. Thin slices of renal cortex were prepared freehand and incubated in 2.7 ml of the phosphate buffered medium described by CROSS and TAGGART[13] which contained 7.4×10^{-5} *M* PAH and 6.0×10^{-6} ^{14}C-NMN. Incubations were carried out in a Dubnoff metabolic shaker at 25 °C under 100% O_2 for 90 min. After incubation, slices were quickly removed from the medium, blotted dry and weighed. Tissue and a 2 ml aliquot of medium were homogenized with 3 ml 10% TCA and brought to a final volume of 10 ml with distilled water.

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[4] M. HORSTER and J. E. LEWY, Am. J. Physiol. *219*, 1061 (1970).

[5] J. K. KIM, G. H. HIRSCH and J. B. HOOK, Pediat. Pediat. Res. *6*, 600 (1972).

[6] G. H. HIRSCH and J. B. HOOK, J. Pharmac. exp. Ther. *171*, 103 (1970).

[7] J. L. ECKER and J. B. HOOK, Biochim. biophys. Acta. *339*, 210 (1974).

[8] J. B. HOOK, Proc. 5th int. Congr. Nephrol. *1*, 6 (1974).

[9] G. H. HIRSCH and J. B. HOOK, Science *165*, 909 (1969).

[10] G. H. HIRSCH and J. B. HOOK, J. Pharmac. exp. Ther. *174*, 152 (1970).

[11] H. V. BRÄUNLICH, K. LUTHER and S. RUDOLPH, Experientia *30*, 1314 (1974).

[12] G. BERNHARDT, H. BRÄUNLICH, C. DIETZE, W. LUNGERSHAUSEN and R. SCHADE, Acta biol. med. germ. *31*, 423 (1973).

[13] R. J. CROSS and J. V. TAGGART, Am. J. Physiol. *161*, 181 (1950).

PAH in tissue and medium supernatants was determined by the method of SMITH et al.[14]. NMN concentration in the supernatant was determined by counting aliquots in modified Bray's solution using a Beckman LS100 liquid scintillation spectrophotometer. Data were presented as a slice to medium (S/M) concentration ratio where S equals mg/g tissue (wet weight) and M equals mg/ml medium.

Renal function was determined in vivo using rats anesthetized with 60 mg/kg sodium pentobarbital. Body temperature was maintained at 33–38 °C using heat lamps. A PE 50 cannula was inserted into the bladder and urine was collected under mineral oil in preweighed vials. Both femoral arteries were cannulated to monitor blood pressure (Statham arterial pressure transducer) and to obtain blood samples. The left femoral vein was cannulated for infusion of a solution containing 1% inulin and 0.5% PAH in normal saline. ^{3}H inulin (0.5 μCi/ml) and ^{14}C PAH (0.5 μCi/ml) were added to the solution and it was infused at 0.018 ml/min using a Harvard infusion pump. A minimum of 1.5 h elapsed from the beginning of the infusion to initiation of urine collection. Three, 30-minute urine samples were taken. Blood (300 μl) was sampled at the middle of each urine collection. ^{14}C-PAH and ^{3}H-inulin in plasma and urine were determined using a Packard Tri-Carb Liquid Scintillation Spectrometer.

Data were analyzed statistically by either Student's *t*-test or randomized complete block analysis of variance[15]. The 0.05 level of probability was used as the criterion of significance.

Effect of PAH and THAM pretreatment on clearance of inulin and PAH and filtration fraction in adult rats

	Control	PAH	THAM
Inulin clearance (ml/min)	1.48±0.13(5)	0.99±0.09(4)[a]	1.35±0.14(4)
PAH clearance (ml/min)	4.47±0.30(5)	3.52±0.28(4)[a]	4.28±0.36(4)
Filtration fraction	0.33±0.01(5)	0.28±0.01(4)[a]	0.31±0.01(4)

Beginning at 55 days of age rats were treated with either 300 mg PAH or 94 mg THAM per 100 g body weight twice daily for 4 days and sacrificed 24 h after the final injection. Filtration fraction was calculated as the ratio of inulin to PAH clearance. Values represent mean ± SE. Numbers in parenthesis designate number of animals tested.

[a] Significantly different from control ($p<0.05$).

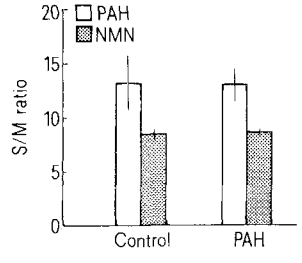

Fig. 1. Effect of PAH pretreatment on the accumulation of PAH and NMN by renal cortical slices. Bars represents mean ± SE of 5 determinations.

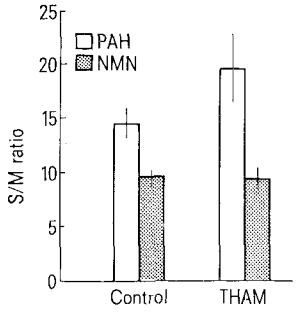

Fig. 2. Effect of THAM pretreatment on the accumulation of PAH and NMN by renal cortical slices. Bars represent mean ± SE of 9 determinations.

Results. Pretreatment of adult rats with PAH had no significant effect on the ability of renal cortical slices to accumulate either PAH or NMN (Figure 1). THAM pretreatment had no effect on the accumulation of NMN but significantly increased PAH (Figure 2). In intact animals PAH pretreatment significantly decreased both PAH (effective renal plasma flow) and inulin (glomerular filtration rate) clearance. THAM pretreatment had no effect on these measurements (Table).

Discussion. BRÄUNLICH et al.[11] observed that pretreatment of 55-day-old rats with 94 mg THAM per 100 g body weight increased the rate of THAM excretion approximately 50%. Similarly, in rats pretreated with 300 mg PAH per 100 g body weight PAH excretion was increased approximately 45%[12]. In both studies, renal transport was estimated as total urinary excretion. These methods failed to take into account alterations in blood flow to the kidney, volumes of distribution or changes in general health of the animal. In addition, large doses of organic acids and bases could result in osmotic diuresis which might influence rates of excretion of these compounds. Therefore, the experiments in this study were undertaken to more carefully evaluate transport capacity in pretreated animals.

The age and treatment schedule were chosen from the cited studies to provide optimum stimulation[11,12]. Administration of PAH produced severe, outward signs of distress in the animal. Violent abdominal contractions were observed. Animals immediately began to drink following injection, presumably because of the osmotic effect of this large amount of PAH. The reaction to THAM pretreatment was less severe. The only effect of pretreatment observed in vitro was an increased accumulation of PAH following THAM. This is obviously not a case of substrate stimulation and may be due to a nonspecific toxic action of THAM on renal tissue comparable to the stimulation of PAH transport following low doses of gentamicin[16] or potassium dichromate[17].

In the intact animal the clearances of PAH and inulin were determined to further investigate the increased in vitro accumulation of PAH following THAM and to evaluate toxicity. The only effect observed was a general diminution of function following PAH treatment, secondary to toxicosis.

In conclusion, these data suggest that large doses of PAH and THAM have no direct effect on renal organic ion transport capacity. Any differences in transport measurement following pretreatment in this study were most likely due to non-specific toxicity.

[14] H. W. SMITH, N. FINKELSTEIN, L. ALIMINOSA, B. CRAWFORD and M. GRABER, J. clin. Invest. *24*, 388 (1945).

[15] R. G. D. STEELE and J. H. TORRIE, *Principles and Procedures of Statistics* (McGraw-Hill, New York 1960).

[16] L. COHEN, R. LAPKIN and G. J. KALOYANIDES, J. Pharmac. exp. Ther. *193*, 264 (1975).

[17] W. O. BERNDT, Toxic. appl. Pharmac. *32*, 40 (1975).