The lipoperoxidation rates are given in Table II. The values with added cofactors correspond well with the enzyme activities shown in Table I, thus reflecting the close connection with the NADPH-depending microsomal electron transport chain. The rate of cofactor-supported lipoperoxidation in human fetal liver was 3 to 5 times greater when compared to fetuses of other mammalian species studied. This is in agreement with the higher content of cytochrome P-450 and higher activities of related enzymes in human fetal liver. Furthermore, it must be taken into consideration that animal fetuses were end-term ones, whereas human fetuses were about 14- to 16-weeks of fetal age, i.e., from the first half of pregnancy.

Although the activation of the lipoperoxidation by ascorbic acid is known to be non-enzymatic[23], the values found rely on the enzyme activities. The reason why the fetal and newborn guinea-pig show less lipoperoxidation activity than to those of other species even after addition of ascorbic acid remains obscure. It may be due to the fact that guinea-pig differs from rat and rabbit in the ascorbic acid metabolism by lacking the microsomal enzyme L-gulonolactone oxidase.

Our results show that fetuses of common laboratory animals have only negligible levels of drug-metabolizing enzymes and cofactor-supported lipoperoxidation. On the other hand, the human fetal liver contains a typical intact electron transport chain and actively supporting lipid peroxidation. The suggested role of lipoperoxidation in drug-induced tissue lesions, e.g. in CCl_4-induced liver injury, permits the speculation that animal fetuses are resistant to those kinds of injuries, whereas the human fetus may develop lipoperoxidation-mediated tissue lesions because of the presence of an intact electron transport chain in liver microsomes.

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The Effect of Thiazol-4-ylmethoxyamine, a Histidine Decarboxylase Inhibitor, on the Development of Morphine Tolerance and Physical Dependance in Mice[1]

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Summary. A new histidine decarboxylase inhibitor, thiazol-4-ylmethoxyamine (TMA), injected into mice in a dose of 100 mg/kg i.p. 48 h before the implantation of a morphine-containing pellet, inhibited the development of morphine tolerance and physical dependence.

General inhibitors of protein synthesis, e.g. cycloheximide, are known to impair the development of morphine tolerance and physical dependence[3,4]. This action is presumably related, at least in part, to a depression of synthesis of enzymes involved in the metabolism of neurotransmitters which mediate these central actions of morphine. There is increasing evidence that histamine (Hm) may be a transmitter in the brain[5], and some recent work appears to implicate Hm in the neural mechanisms of morphine tolerance and physical dependence[6–8]. If this is so, then the specific and potent histidine decarboxylase inhibitor thiazol-4-ylmethoxyamine (TMA)[9,10], which can cross the blood-brain barrier, should have actions rather similar to cycloheximide in this respect.

Materials and methods. Mice (WHT/Ht strain; both sexes, weighing 25–40 g, in roughly equal numbers) were

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Table I. Effect of TMA on the analgesic action of morphine in naive and oependent mice[a]

Treatment	Naive ($n=25$)		Dependent ($n=24$)		Slope Ratio	Potency Ratio	$f_{P.R.}$
	Slope function	AD_{50} (mg/kg)	Slope function	AD_{50} (mg/kg)		(P. R.)	
Control group	2.02 (2.46–1.66)	6.85 (8.84–5.31)	2.27 (3.97–1.30)	17.5 (39.8–7.7)	1.12 (2.02–0.68)	2.55 (4.72–1.38)	1.85
TMA group	1.23 (1.62–1.08)	9.20 (11.73–7.22)	2.02 (3.22–1.30)	11.00 (18.15–6.67)	1.64 (2.83–0.95)	1.20[b] (2.1–0.69)	1.75

[a] The potency is expressed as the median analgesic dose (AD_{50}) of morphine after morphine-containing and blank pellet implantation. Figures in parantheses denote 95% confidence limits.
[b] As the P. R. is smaller than the $f_{P.R.}$, the 2 AD_{50} values are not significantly different.
Calculations according to the methods of LITCHFIELD and WILCOXON[17]

Table II. Effect on TMA on brain level of Hm and uptake of morphine in naive and dependent mice

		μg Morphine/g brain[a] ± SEM	ng Histamine/g[b] brain ± SEM
Control group	Naive	1.34±0.04 (5)	161.71±8.3 (7)
	Dependent	1.27±0.08 (4)	170.44±11.06 (6)
TMA group	Naive	1.45±0.20 (4)	173.94±5.52 (5)
	Dependent	1.63±0.03 (5)[c]	170.20±9.62 (4)

Numbers of mice in brackets.
[a] 30 min after 60 mg/kg morphine sulphate, i.p.; and 3.5 h after removal of the pellet implanted for a day. TMA was administered 48 h before the implantation of the pellet.
[b] TMA was injected 48 h before the implantation of the pellet. 24 h after the implantation of the pellet, the mice were decapitated and the brain histamine estimated.
[c] $p<0.01$, compared with its corresponding control (Student's t-test).

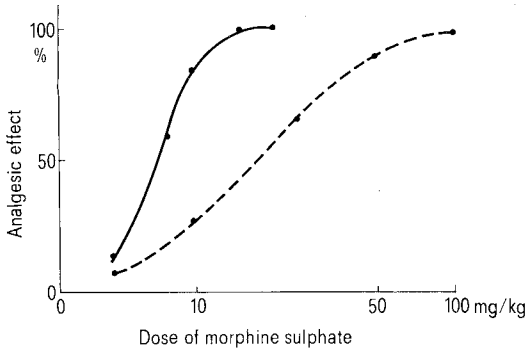

Fig. 1. Log-dose response curves of the analgesic effect of morphine in naive (solid line) and dependent (broken line) mice. Each point represents the mean of 4 or 5 measurements.

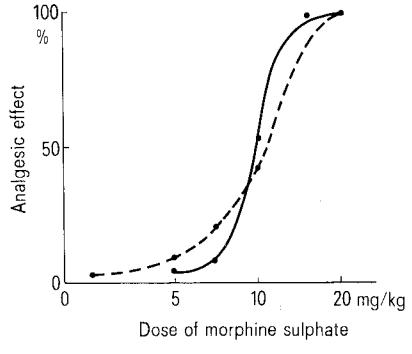

Fig. 2. Log-dose response curves of the analgesic effect of morphine in naive (solid line) and dependent (broken line) mice treated with TMA. Each point represents the mean of 4 or 5 measurements.

made morphine-dependent by the implantation of a new type of morphine-containing pellet[11]; these are referred to as dependent mice. Non-dependent mice, given a blank pellet without morphine, are referred to as naive mice. TMA (100 mg/kg) was injected i.p. 48 h before pellet implantation, as a brain Hm depletion is said to be maximal after about 72 h with recovery to normal over the next 2–3 days[10]. 24 h after implantation, the pellet was removed, and 3 h later mice were submitted to the tail-flick test[12] in order to measure the antinociceptive effect of various doses of morphine sulphate (2.5–20 mg/kg). Physical dependence was assessed in the same mice by giving a naloxone challenge (0.5–200 mg/kg[13]) 1 h after the morphine sulphate administration. The percentage of mice leaping off a raised platform in the 15 min following the dose of naloxone was used as the sole criterion for assessment of physical dependence[14].

Brain morphine and Hm were estimated by the fluorometric method of KUPFERBERG et al.[15] and HÅKANSON et al.[16] respectively (Aminco-Bowman SPF).

The well-known procedure of LITCHFIELD and WILCOXON[17] was used to estimate the ED_{50} and AD_{50} values and their 95% confidence limits. The degree of morphine tolerance was measured by the increase in AD_{50}, and the degree of physical dependence by the increase in naloxone ED_{50}.

Results. Table I shows that the difference between AD_{50} values for morphine in the dependent and naive groups treated with TMA is statistically non-significant (11.0 and 9.2 mg/kg; potency ratio 1.2). In the control groups not treated with TMA, the corresponding values are 17.5 and 6.85 (potency ratio 2.55), indicating the development of significant tolerance. The dose-response curves on which these data are based are illustrated in Figures 1 and 2. TMA clearly inhibits the development of morphine tolerance. The whole brain uptake of morphine was shown to be significantly higher ($p< 0.01$) in dependent TMA-treated mice than in dependent control mice, but not in comparison with TMA-treated naive mice (Table II). This finding cannot, therefore, provide a convincing explanation for the observed inhibition of tolerance development.

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Table III. Naloxone-precipitated jumping in dependent mice[a]

Control group (n=20)		TMA group (n=25)		Slope Ratio	Potency Ratio
Slope function	ED_{50} (mg/kg)	Slope function	ED_{50} (mg/kg)		(P. R.)
1.22 (1.59–0.94)	0.94 (1.18–0.75)	1.54 (1.93–1.23)	1.45 (2.12–0.99)	1.26 (1.76–0.9)	1.54 (2.38–1.00)

[a] The potency is expressed as the median effective dose (ED_{50}) of naloxone producing jumping in morphine-dependent mice. Figures in parentheses denote 95% confidence limits (calculations according to LITCHFIELD and WILCOXON[17]).

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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