suggested that the drug-metabolizing enzymes are rate-limiting in the detoxification of aflatoxin[5]. The acute toxicity is inversely related to the total activity of drug-metabolizing system. The present experiment lends support to this suggestion.

A variety of compounds have been tested in cold environment in animals[6]. A typical experiment of immediate relevance is the reported increased susceptibility of mouse liver to carbon tetrachloride (CCl_4) toxicity under cold environment[8]. The behaviour of CCl_4 is found to be exactly opposite to that of aflatoxin in a given situation[5]. It is also believed that CCl_4 requires activation before being toxic[9,10]. This metabolic basis appears to be responsible for the increased susceptibility of mouse liver to CCl_4 under cold environment. Investigations of this nature thus help to explain the mechanism of action of a compound under study.

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Interference with Histamine and Imidazole Acetic Acid Metabolism by Salicylates: a Possible Contribution to Salicylate Analgesic Activity?

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Summary. In man, rats and mice, the urinary excretion of the histamine and L-histidine metabolite, imidazole acetic acid, is increased and that of the conjugated metabolite, ribosylimidazole acetic acid, decreased by small doses of salicylates. In contrast to salicylates, other non-salicylate anti-inflammatory drugs, indomethacin, phenylbutazone, phenacetin and acetaminophen do not influence the excretion of the urinary metabolites of histamine and L-histidine. Since imidazole acetic acid is reported to have analgesic and narcotic activity, there is the inference that the analgesic properties of salicylate might be due in part to interference in imidazole acetic acid metabolism.

In an earlier study, we reported that in man and rat administration of salicylates results in a reduction in the urinary excretion of the histidine and histamine metabolite, ribosylimidazole acetic acid[1]. This reduction is accompanied by a corresponding increase in the excretion of free imidazole acetic acid. Additional studies in vitro have indicated that salicylates inhibit (50% inhibition at 0.2 m*M*) imidazoleacetate phosphoribosyl transferase, the enzyme responsible for the ribosylation of imidazoleacetate in vivo[2]. Since it has been reported that imidazole acetic acid has analgesic and narcotic activity in mouse[3,4], accumulation of imidazole acetic acid during salicylate therapy might contribute to the analgesic action of these drugs. As part of a continuing study to explore this possibility, this report compares the effect of salicylates on imidazole acetic acid metabolism in mouse with that in rat and man.

Materials and methods. Histamine (2-ring-^{14}C) was purchased from Amersham/Searle (Illinois). The ^{3}H-imidazole acetic acid was prepared from unlabeled material by catalytic exchange with tritium gas (New England Nuclear Corp.) and was purified by thin layer chromatography[1].

Human subjects (18–54 years of age) included 2 normal volunteers and 9 patients with mild hypertension or Raynaud's disease who were receiving no drugs except aspirin. Aspirin was administered 4 times daily for 1 week before the injection of ^{14}C-labeled histamine. In control studies, aspirin treatment was suspended for 1 week before injection of ^{14}C-histamine. All subjects were fully informed of the nature of the experiments and were free to discontinue their participation in the study if they wished. Animals were kept in glass metabolic cages (1 rat per cage, 5 mice per cage) with free access to food and water. Food was withdrawn during the period of urine collection. Aspirin and other drugs were administered orally by stomach tube in a single or repeated (q.i.d.) doses.

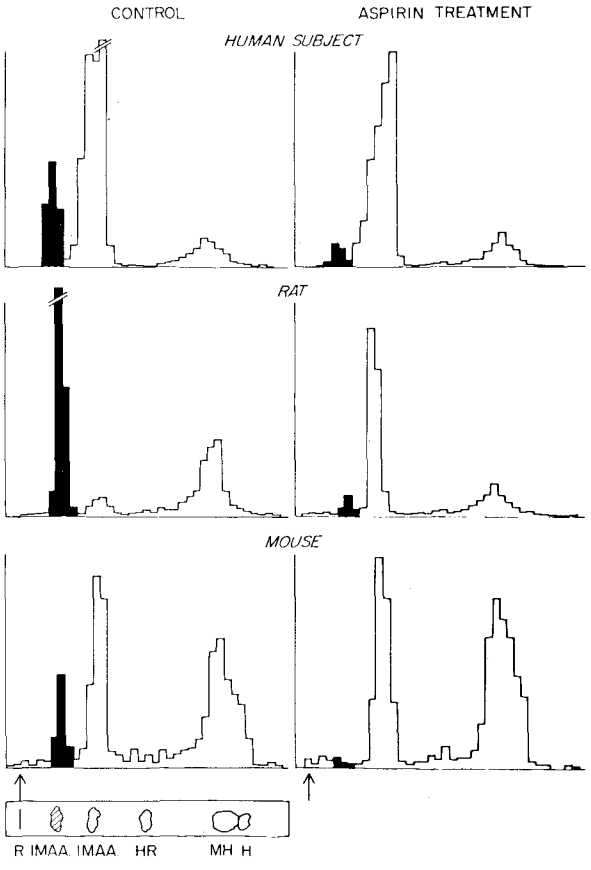
^{14}C-Histamine (5 μCi/kg in man or 25 μCi/kg in rat and mouse) and ^{3}H-imidazole acetic acid (20 μCi/kg) were given i.v. 2 h after the administration of drug. Urine was then collected for 6 h periods and frozen. Aliquots (10 μl) of urine were assayed for labeled histamine metabolites by thin layer chromatography on chromatograms of cellulose powder on flexible 20 × 5 cm plastic sheets in butanol:ethanol:ammonia (80:10:30 parts by volume) solvent for 90 min and by isotope dilution-derivative techniques[1,2]. As discussed elsewhere[1], there is close agreement in values obtained by the two procedures.

Results. In urine of all species, 3 major peaks of radioactivity – ribosylimidazole acetic acid, unconjugated acids (imidazole and methylimidazole acetic acid), and methylhistamine – were identified on thin layer chromatograms after the injection of ^{14}C-histamine (Figure). 2 major peaks – ribosylimidazole acetic acid and imidazole acetic acid – were identified after the injection of ^{3}H-imidazole acetic acid (not shown in the Figure). Upon treatment with aspirin, the ribosylimidazole acetic acid peak disappeared in all species (Figure) and reappeared once aspirin treatment was stopped.

In humans, after treatment with 4 × 900 mg or 4 × 600 mg aspirin daily, the excretion of ^{14}C-ribosylimidazole acetic acid decreased from 18% to 4% (percent of injected label). The output of unconjugated acids increased by a corresponding amount. The excretion of histamine and methylhistamine was unchanged (Table). In all subjects, ribosylimidazole acetic acid reappeared in urine when aspirin was stopped.

In rats, the ribosyl derivative of imidazole acetic acid accounted for the major part of the label in urine (Figure and Table). Treatment with as little as 25 mg/kg aspirin reduced urine excretion of the ribosyl conjugate by 50–60%; higher doses of aspirin produced an even greater reduction[1]. As in humans, the excretion of unconjugated acid increased by a corresponding amount while that of methylhistamine and histamine was unchanged (Table).

Nonsalicylate anti-inflammatory drugs, such as phenacetin, acetaminophen, phenylbutazone and indomethacin, were without effect (Table)[1].



In mouse, the extent of conjugation was less than that in rat, but, as in rat and man, the conjugation was blocked by aspirin administration (Figure and Table). Methylhistamine was the major urinary metabolite in this species (Table).

The studies with ^{3}H-imidazole acetic acid showed that in rat the acid was excreted in urine almost exclusively (> 94%) as ^{3}H-ribosylimidazole acetic acid; in mouse, the acid was only partly (53%) converted to the ribosyl conjugate (Table). No other metabolite of the acid was detected in urine. The formation of ^{3}H-ribosylimidazole acetic acid was blocked completely by aspirin treatment (Table). After the injection of ^{3}H-imidazole acetic acid, the label distributed rapidly and uniformly in all tissues including brain.

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Effect of aspirin treatment on excretion of labeled metabolites of ^{14}C-histamine in 3 species. The figures show chromatograms of urine collected 6 h following injection of ^{14}C-histamine before and during aspirin treatment. Urine, 10 µl, was chromatographed on cellulose powder plates. Segments, 2.5 mm, were assayed for radioactivity. 3 major peaks – 1. ribosylimidazole acetic acid (RIMAA); 2. imidazole (IMAA) and methylimidazole (MeIMAA) acetic acid, and 3. methylhistamine (MH) and histamine (H) – were identified by comparison with known standards. Assays by isotope dilution and derivatization procedures indicated that the 3rd peak was largely methylhistamine. Ribosylhistamine (HR) was not detected in urine in all species. In all species, the ribosylimidazole acetic acid (shaded column) was reduced during aspirin treatment

Effect of aspirin and other anti-inflammatory agents on excretion of ^{14}C-histamine and ^{3}H-imidazole acetic acid metabolites in man, rat and mouse

Treatment	*n*	Label (%) excreted in urine as					
		Ribosylimidazole acetic acid		Free imidazole and methylimidazole acetic acid		Methylhistamine and histamine[a]	
		^{14}C	(^{3}H)	^{14}C	(^{3}H)	^{14}C	(^{3}H)
Human subjects							
Control	9	18 ± 1		67 ± 2[b]		14 ± 1	
Aspirin (4 × 900 mg/day)	4	4 ± 1		83 ± 1		12 ± 1	
Aspirin (4 × 600 mg/day)	3	4 ± 1		87 ± 2		10 ± 2	
Rat[c]							
Control	5	57 ± 8	(94)	6 ± 2	(6)	37 ± 10	(—)
Aspirin (4 × 300 mg/kg)	4	4 ± 3	(4)	54 ± 15	(95)	39 ± 16	(—)
Salicylate 4 × 300 mg/kg)	2	4		59		31	
Phenacetin (4 × 100 mg/kg)	2	62		9		25	
Acetaminophen (4 × 200 mg/kg	2	62		6		25	
Phenylbutazone (4 × 200 mg/kg)	2	53		8		34	
Indomethacin (4 × 5 mg/kg)	3	52 ± 5		13 ± 5		31 ± 2	
Mouse							
Control	10	13	(53 ± 5)	37	(47 ± 5)	50	(—)
Aspirin (4 × 300 mg/kg)	10	<3	(8 ± 4)	40	(91 ± 2)	57	(—)

Values were obtained by chromatographic procedures described in Materials and Methods and are the mean ± SEM or the average of duplicate experiments where applicable. Values in parenthesis are for tritium-labeled metabolites from ^{3}H-imidazole acetic acid. [a] Assay by isotope dilution-derivative procedures indicated that ^{14}C-histamine constituted 2–3% of the label in urine as noted in the Figure. [b] Isotope dilution assays indicated that 82% of label in this fraction was methylimidazole acetic acid. [c] Includes data from Beaven *et al.*[1].

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