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Influence of sex and Freund's adjuvant on liver *N*-acetyltransferase activity and elimination of sulphadimidine in urine of rats

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Two distinct acetylation phenotypes, characterized by high or low percentage of acetylated product of isoniazid and sulphadimidine eliminated in urine, can be distinguished in man [1–6] and rabbits [7–10]. The difference between them is believed to be due to differences in the activity of *N*-acetyltransferase [10–16]. In these two species no sex difference in the two phenotypes exists, but in rats we have found [17] that the high elimination of acetylsulphadimidine (Ac-S) occurs in females (about 75 per cent of sulphadimidine in urine is present as Ac-S), and low elimination in males (about 46 per cent). The aim of this work was to investigate whether this sex difference is also associated with corresponding differences in liver *N*-acetyltransferase activity. It has been found in rats [18] that Freund's adjuvant which is a potent stimulant of the reticuloendothelial system, presumably the main site of drug acetylation [19], increases the percentage of Ac-S in urine of males, but not of females. Similar elevation of Ac-S only in males was observed also after castration and treatment by estrogen [20]. Therefore, it was of interest to find out whether these changes in males were also accompanied by corresponding changes in enzyme activity.

Animals and experimental conditions. Random-bred albino rats (10 to 16 weeks old) obtained from the Institute of Organic Chemistry and Biochemistry, Prague, were used. They were divided into five groups (see Table 1) as follows: (1) untreated females; (2) untreated males; (3) castrated males: castration was performed when they were 6 weeks old and sulphadimidine was administered 10 weeks later; (4) estrogen-treated males: oestradiol dipropionate in oil solution (Agofollin, Spofa) was given subcutaneously in five daily doses, each containing 10,000 i.u. of the hormone, and sulphadimidine was then administered 2 days after the last dose of estrogen; (5) Freund's adjuvant- (FA-) treated males: 0.1 ml of complete FA (5 mg of *Mycobacterium tuberculosis*, strain H37Rv/1 ml of mineral oil) was injected into the skin of the foot pad and then sulphadimidine was given 21 days later when effect of the adjuvant was fully developed [21, 22].

Sulphadimidine was always administered intravenously in a dose of 40 mg/kg and in a volume of 0.2 ml/100 g body wt. Following the procedure described elsewhere [17], urine samples collected during a 24-hr interval after drug administration were analysed and sulphadimidine determined according to the method of Varley [23]. The ratio (percentage) of Ac-S to the total sulphadimidine eliminated in the urine was then calculated and statistically evaluated.

Preparation of liver cytosol. Since cells of the liver reticuloendothelial system have been shown to represent the most important site of drug acetylation [19], only this tissue was used for preparation of the cytosol. Fresh liver tissue was obtained from rats immediately after sacrifice. All samples were chilled and weighed and then homogenized at 4° in 5–10 vol of Sörensen phosphate buffer (pH 7.4) in a Teflon homogenizer. Homogenates were centrifuged at 105,000 *g* for 50 min in a Spinco ultracentrifuge. The resulting supernatant was used in the enzyme assay which allowed the estimation of total *N*-acetyltransferase activity in the absence of competing microsomal enzymes.

Incubation procedure. The method of Jenne [16] was modified to suit our purpose. The incubation mixture contained the following in a final vol of 2.0 ml: 2 m-mole of sulphadimidine as substrate, 0.5 m-mole of aqueous acetyl-CoA and liver cytosol suitably diluted in 0.2M Sörensen phosphate buffer (pH 7.4) to contain 10 mg of supernatant protein per ml. The reaction was initiated by the addition of liver cytosol and allowed to proceed at 37° for 15 min. The reaction was terminated by adding 4.9 ml of 8% (w/v) trichloracetic acid. Enzyme activity was estimated from the amount of Ac-S produced in the incubation mixture [23]. Protein concentration was determined by the technique of Lowry [24] using bovine serum albumin as a standard. Enzyme activity was expressed as μmoles of substrate acetylated per mg of supernatant protein per 15 min incubation.

The results are summarized in Table 1. The percentage of Ac-S eliminated by untreated animals corresponds well with our previous data [17, 18] showing that females eliminate a high and males a low percentage of Ac-S. The difference between untreated females and males is statistically highly significant ($t_{(16)} = 15.96$, $p \ll 0.01$). The increase in Ac-S elimination in all the groups of treated males is also statistically highly significant: $t_{(13)} = 5.12$, $p < 0.01$ for castration; $t_{(14)} = 13.49$, $p \ll 0.01$, for estrogen treatment; and $t_{(13)} = 14.12$, $p \ll 0.01$ for FA treatment.

The activity of *N*-acetyltransferase is about three times higher in untreated females than in males, the difference being statistically highly significant ($t_{(16)} = 18.16$, $p < 0.01$). However, no increase of *N*-acetyltransferase activity was brought about by castration ($t_{(13)} = 1.33$, $p > 0.10$), estrogen treatment ($t_{(14)} = 1.38$, $p > 0.10$) or administration of FA ($t_{(13)} = 0.47$, $p > 0.50$).

It should be pointed out that while all experimental treatments employed increase the percentage of Ac-S in the urine of males, they do not affect the activity of liver *N*-acetyltransferase. Obviously, the proportion of metabolized and unchanged sulphadimidine in urine is influenced by various mechanisms. The role of *N*-acetyltransferase activity seems to be decisive mainly under normal conditions, i.e. in untreated animals. The nature and interaction of other mechanisms is rather poorly understood at present but our preliminary data (unpublished results) suggest the importance of differences in the distribution of sulphadimi-

Table 1. Percentage of eliminated acetylsulphadimidine in urine and activity of *N*-acetyltransferase in liver of untreated and castrated, estrogen-and Freund's adjuvant-treated rats

	Females	Males			
	Untreated	Untreated	Castration	Oestradiol‡ dipropionate	Freund's§ adjuvant
Number of animals	10	8	7	8	7
Percentage of eliminated Ac-S	75.5 ± 2.73*	46.8 ± 3.14	65.7 ± 8.43	69.6 ± 2.50	73.4 ± 3.45
N-acetyltransferase activity†	29.5 ± 1.73	10.8 ± 1.50	9.8 ± 0.84	12.0 ± 1.40	11.2 ± 1.36

* 95% limits of confidence.

† Enzyme activity is expressed as μmoles of substrate acetylated by 1 mg of supernatant protein per 15 min of incubation.

‡ Five daily subcutaneous doses containing 10,000 i.u. of the hormone each, were applied.

§ Intradermal injection of 0.1 ml of an adjuvant mixture (5 mg of *Mycobacterium tuberculosis*/ml of mineral oil) into the foot pad, was applied.

dine in the body of both sexes and in the body of untreated vs treated males.

These findings also bear on the question of the proper nomenclature of the acetylation status of an organism. Even when it is true that the acetylation status in man and rabbits is not linked to the sex as it is in rats, it appears for reasons given above that the frequently used terms "rapid" or "slow acetylator", based on fractional amount of metabolite present in urine might not be, generally, the most appropriate ones. It is therefore suggested that unless the activity of *N*-acetyltransferase is determined directly—this cannot be done routinely either in man or animals—the terms "high" or "low eliminator of Ac-S" should be preferred.

Summary. Relatively high activity of liver *N*-acetyltransferase was found in control, untreated females and on the other hand low enzyme activity in males of rats. This difference is accompanied with relatively high percentage of Ac-S eliminated in urine of females whereas it is low in males. Castration, estrogen and Freund's adjuvant treatment increase the percentage of Ac-S in males, but do not affect the activity of *N*-acetyltransferase.

Institute of Pharmacology, Czechoslovak Academy of Sciences, 128 00 Prague 2, Czechoslovakia

ZDENĚK ZÍDEK
MARTA FRIEBOVÁ
IVO JANKŮ
JIŘÍ ELIS

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