## Studies on the different metabolic pathways of antipyrine as a tool in the assessment of the activity of different drug metabolizing enzyme systems in man

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Antipyrine plasma half-life or clearance values are widely used to assess changes in the activity of hepatic mono-oxygenases in man. However these parameters do not represent an absolute measure of an individual's capacity to metabolize drugs, since poor correlations have been observed with the clearances of many other drugs that are mainly eliminated by oxidation. It has been suggested that these discrepancies are due to the qualitative and quantitative heterogeneity of the mixed function oxidase system. On the other hand antipyrine metabolism is rather complicated since at least four phase I metabolites have been identified: 4-hydroxy-antipyrine, norantipyrine, 3-hydroxymethyl-antipyrine and 3-carboxy-antipyrine. Methods were developed to determine these compounds quantitatively in urine by high-pressure liquid chromatography or gas chromatography (Danhof, de Groot-van der Vis & Breimer, 1979; Danhof, de Boer, de Groot-van der Vis & Breimer, 1979). Following oral administration of antipyrine (500 mg) to healthy volunteers 3.3% of the dose was excreted as unchanged drug, 28.5% as 4-hydroxy-antipyrine, 16.5% as norantipyrine, 35.1% as 3-hydroxymethyl-antipyrine and 3.3% as 3-carboxy-antipyrine (mean values; 52 h urine). These cumulative amounts of excretion products were not significantly different at 250 and 1000 mg dose levels in the same panel of volunteers (Danhof & Breimer, unpublished). The major part of the metabolites was excreted as glucuronides.

Evidence was obtained in rats that different types of hepatic monooxygenases are involved in the formation of antipyrine metabolites: after 3-methylcholanthrene treatment a significant increase in 4-hydroxy-antipyrine formation occurred, whereas 3-hydroxymethyl-antipyrine formation was significantly decreased (Danhof, Krom & Breimer, 1979). Phenobarbitone pretreatment did not result in a change of antipyrine metabolite ratios in rats. In healthy volunteers, however, pentobarbitone treatment (100 mg for 8 days) resulted in a selective increase in norantipyrine formation and a decrease in 3-hydroxymethyl-antipyrine formation, whereas the excreted amount of 4-hydroxy-antipyrine remained almost unchanged. Preliminary results in patients with liver disease indicate that antipyrine metabolite ratios are quite different in individual cases compared to healthy men.

Studies on the different metabolic pathways of antipyrine may be quite important as a tool in the assessment of the activity of different oxidative drug metabolizing enzyme systems in man.

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## Inhibition of aminopyrine demethylation and binding to cytochrome P-450 by its main metabolites in rat liver microsomes

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Aminopyrine (DMAP) is demethylated by two successive *N*-demethylations to monomethyl-4-aminoantipyrine (MMAP) and 4-aminoantipyrine (AAP), respectively. This reaction can be estimated *in vitro* by measuring formaldehyde formation and is frequently used to determine the hepatic monooxygenase activity. It is possible that aminopyrine metabolites will inhibit DMAP demethylation, if they are formed in sufficient quantities. Goromaru, Matsuyama, Noda & Iguchi (1978) demonstrated that, in man, the MMAP plasma concentration achieved a level equal to or higher than the DMAP plasma concentration.

In our study, microsomes of livers from male Wistar rats (250 g) were suspended in a phosphate buffer (50 mM and pH 7.4) containing EDTA (0.1 mM). Incubation (5 min at 37°C) of dimethyl-[$^{14}$C]-aminoanti-