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The tissue distribution and metabolism of amidopyrine in the rat and pregnant and non-pregnant rabbit

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The tissue distribution of amidopyrine and its metabolites 4-amino-antipyrine and *N*-acetyl-4-amino-antipyrine has been determined at intervals following oral doses of 300 mg/kg of amidopyrine in pregnant, non-pregnant and foetal rabbits, and also in adult non-pregnant rats. Except in lung, their concentration was higher in the normal rabbit than the rat. In both species amidopyrine levels were maximal at 2 hr, but the metabolite levels varied with tissue, species and time interval. The dominant pathways were demethylation in the rabbit and acetylation in the rat. Plasma and tissue levels of amidopyrine were higher in non-pregnant than pregnant rabbits, whereas those of the metabolites varied. Studies indicate that 25-day, but not 14-day foetuses may metabolize amidopyrine.

SPECIES differences in the metabolism of drugs is well known (Burns, Ross & others, 1953; Brodie, Burns & others, 1953; Perel, Chen & others, 1961), so too is the fact that drug metabolism varies with pregnancy and in neo-nates (Brodie & Maickel, 1962; Hartiala, Pulkkinen & Rauramo, 1963; Lessel & Cliffe, 1964).

In a systematic investigation on drug metabolism and tissue distribution (Crema & Berté, 1960; Mascherpa, 1963; Mascherpa & Berté, 1966), the behaviour of amidopyrine in different species, and in pregnancy and pre-natality, was examined because it is still used as an analgesic and antipyretic; its main metabolic pathway is known and involves two serial steps (demethylation to 4-aminoantipyrine and acetylation of this compound to *N*-acetyl-4-aminoantipyrine), other metabolites are also present with or without ring cleavage, and its physiological disposition is known in man and dog. The present paper describes the tissue distribution of amidopyrine and two metabolic products (4-aminoantipyrine and *N*-acetyl-4-aminoantipyrine) in the pregnant rabbit and in the rabbit foetus, and also in the adult non-pregnant rat and rabbit at different times after drug administration. The metabolic transformations in some rabbit adult and foetal tissues have also be examined.

Experimental

Tissue distribution of amidopyrine. Fifty-four adult female Wistar rats (220 ±5 g), 12 adult female Dutch rabbits (2·0 ±0·18 kg) and 12 female Dutch rabbits at the 25th day of pregnancy (2·6 ±0·24 kg) were used; amidopyrine was administered orally by stomach tube to the fasting animals at the dose of 300 mg/kg. The animals were killed by bleeding 1, 2, or 4 hr after drug administration and the lung, liver, kidney, brain, muscle, and placenta were immediately homogenized for 5 min with saline solution (200 mg of tissue/ml of homogenate). Amniotic fluid and heparinized blood were centrifuged for 20 min at 2,000 rev/min. The concentration of amidopyrine, 4-aminoantipyrine and *N*-acetyl-4-aminoantipyrine in the biological materials were estimated by the method of Brodie & Axelrod (1950).

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Amidopyrine metabolism. The organs of 12 pregnant Dutch rabbits (2·8 ±0·4 kg) were used; the animals were killed by bleeding at the 25th day of pregnancy and the kidney, liver, lung and brain were removed immediately from mother and foetus. The organs were homogenized with 3·3 volumes of potassium chloride (1·49% for the brain and 1·15% for the other organs) using a Potter type homogenizer. The homogenates (corresponding to 3·0 g of brain and 1·5 g of the other organs) were centrifuged at 9,000 rev/min for 15 min. The supernatants were mixed with 0·4 μmole NADP, 20 μmole glucose 6-phosphate, 50 μmole nicotinamide, 0·1 ml of M potassium chloride solution, 0·1 ml of 7·14% magnesium chloride solution, 0·5 ml of amidopyrine solution (600 μg/ml) and phosphate buffer (pH 7·4) to a final volume of 6 ml. The mixtures were incubated at 37° in an atmosphere of oxygen and shaken at 50 cycles/min. Every 15 min, for 1 hr, a sample of each organ was removed from the shaking incubator and stored at −10°.

The determination of amidopyrine, 4-aminoantipyrine and *N*-acetyl-4-aminoantipyrine was made (Brodie & Axelrod, 1960). The extinction was measured against a blank containing buffer solution instead of supernatant from the homogenates.

Similar experiments were made using the organs from five foetuses at the 14th day.

Results and discussion

The difference in the species in distribution and metabolism of amidopyrine is seen in Fig. 1. With exception of lung, the concentrations of

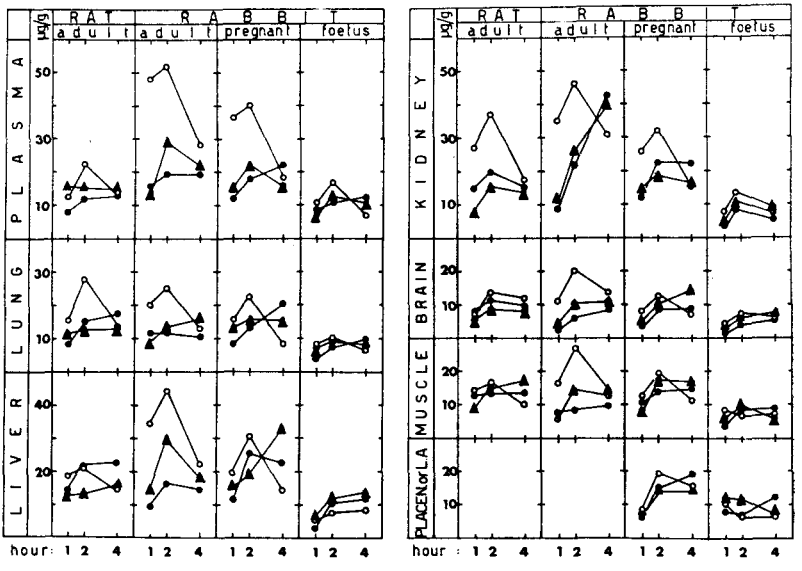

FIG. 1. Distribution (μg/g, on ordinate) of amidopyrine (○—○), 4-aminoantipyrine (●—●) and *N*-acetyl-4-aminoantipyrine (▲—▲), at 1, 2, and 4 hr (on abscissae) after oral administration of 300 mg/kg of amidopyrine to non-pregnant adult rats and rabbits and to rabbits on the 25th day of pregnancy, Placen. = placenta; L.A. = amniotic fluid.

the substance are much higher in organs of the normal rabbit than in the rat. In both animals the amidopyrine concentrations of the organs have a maximum value at the second hour, whereas the 4-aminoantipyrine and the *N*-acetyl-4-aminoantipyrine distribution differs greatly in the various tissues at the different examination times. Also, except in kidney, the acetylated metabolite concentrations are higher in rat than in rabbit, in spite of the lower amidopyrine concentration in plasma. Furthermore, with the exception of plasma and muscle, the 4-aminoantipyrine/*N*-acetyl-4-aminoantipyrine ratio concentration is reversed in rabbit compared to the rat. These data confirm the high activities of demethylation in rabbit and acetylation in rat.

The distribution of amidopyrine and its two metabolites in the tissues including the foetus of the 25-day pregnant rabbit is also shown in Fig. 1. The plasma and tissue levels of the drug are higher in non-pregnant than in the pregnant rabbit, while the concentrations of the two metabolites in the various organs show many differences at the different times of examination. The diffusibility of amidopyrine across the placenta to the foetus is indicated by the presence of the drug in the foetal tissues, although the concentrations are constantly lower than those of the mother. The presence of metabolic products in the organs of the foetus may be due to maternal metabolism, or to placental metabolic activity, or to foetal drug metabolism. To explain this last possibility, we have investigated the amidopyrine metabolism by some organs from mother and 25 day foetus, *in vitro* as indicated in Fig. 2. These foetal tissues can metabolize the

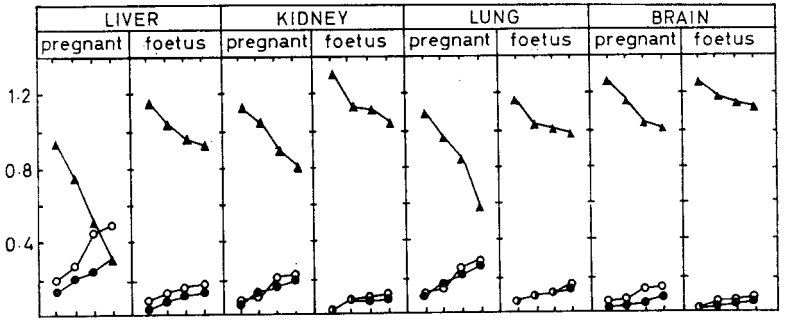

FIG. 2. Metabolism *in vitro* of amidopyrine, for 1 hr at 15 min periods (on abscissae) after substrate activation. The ordinate shows the μmole of amidopyrine (initial value = 1·29 μmole) (▲—▲), 4-aminoantipyrine (●—●) and *N*-acetyl-4-aminoantipyrine (○—○). The data relate to organs from rabbits on 25th day of pregnancy.

amidopyrine although their activity is much lower than that of the mother's organs. On the contrary, no metabolic activity appeared in the organs of 14-day foetuses.

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