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N-demethylation Activity of Renal and Hepatic Subcellular Fractions: An Interspecies Comparison

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p. 82 (Material and Methods)

Demethylation activity assay

Demethylation activity of 9 000 x g supernatant fractions of tissues was estimated by the modified method of Brookman and Kourounakis (1977) using aminopyrine as the substrate. 0.1 ml of 9 000 x g supernatant fraction sample representing 2.5 mg of protein was added to 1.4 ml of an incubation mixture containing (mmol/l): 0.86 NADP, 10.70 glucose-6-phosphate, 5.40 $MgCl_2$, 0.36 aminopyrine in 110 mmol/l TRIS-HCl buffer pH (7.4). The samples were incubated at 37 °C for 90 min, then the reaction was stopped by adding 1.0 ml of 0.6 mol/l trichloracetic acid. Reaction controls for each sample, to which trichloracetic acid had been added before incubation for arresting the enzymatic reaction, were run in an identical manner. After centrifugation at 3 000 x g for 10 min, 1.0 ml of supernatant was used for the determination of formed formaldehyde by Nash reaction (Nash 1953). Samples were neutralized by adding 0.5 ml of 0.3 mol/l sodium hydroxide, then 1.5 ml of reagent was added consisting of 2.0 mol/l ammonium acetate, 0.02 mol/l acetylacetone, and 0.05 mol/l acetic acid. After incubation at 37 °C for 40 min, the absorbance of samples at 412 nm was measured with a reference sample prepared in the same manner with water instead of the incubation medium. The calibration curve for colorimetric assay was prepared with standard formaldehyde solutions in distilled water handled as the samples; it was linear in the range of concentration from 0.01 to 0.20 mmol/l.

p. 85 (Discussion)

With aminopyrine as the MFO-system substrate, Litterst *et al.* (1975) obtained relative demethylation activity values of renal microsome preparations of the rat, rabbit, and guinea-pig about 5 % for all the species tested. With respect to the investigation of Navran and Louis-Ferdinand (1975), who reported a considerable demethylation activity not only in the microsome fraction, but also in the cytosol fraction of the rat renal tissue, it may be considered that the lower demethylation activity in the microsome preparation could be due to a lack of cytosol demethylation activity present in the 9 000 x g supernatant fraction.

p. 84 (Results)

Table 1
Demethylation activity of 9000 x g supernatant fraction of kidney and liver

Species	Demethylation activity (nmol HCHO/mg prot./min)		Relative demethylation activity in the kidney (expressed in % of liver activity
	Kidney	Liver	Kidney
Rat	0.20 ± 0.02	0.45 ± 0.13	44.4 ± 12.4
Rabbit	0.14 ± 0.05	0.30 ± 0.10	46.7 ± 10.1
Guinea-pig	0.11 ± 0.02	0.68 ± 0.14	16.2 ± 5.9