***In Vitro* Inhibition Study of Diphenylhydantoin upon Rat Liver Enzymes Concerned in Folic Acid Metabolism**

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NARISAWA, K., HONDA, Y., YOSHIDA, T. and ARAKAWA, Ts. *In Vitro Inhibition Study of Diphenylhydantoin upon Rat Liver Enzymes Concerned in Folic Acid Metabolism*. Tohoku J. exp. Med., 1972, 106 (3), 249-251 — Results of *in vitro* study using rat liver homogenates revealed that there was no inhibitory effect of diphenylhydantoin (50 γ per ml of the reaction mixture) upon histidase, urocanase, formiminotransferase, N^{5,10}-methylenetetrahydrofolate reductase, formyltetrahydrofolate synthetase, serine-hydroxymethylase and glycine cleavage enzyme. ——— diphenylhydantoin; folic acid

In 1965 Hamfelt and Wilmanns reported that diphenylhydantoin, phenobarbital, or primidone had no inhibitory action on dihydrofolate reductase, methylene tetrahydrofolate dehydrogenase or formyltetrahydrofolate synthetase, which were extracted from an acetone-dried powder of pigeon liver.

The results of present investigation revealed no inhibitory action of diphenylhydantoin on the enzyme systems concerned in folic acid metabolism in rat liver, such as histidase, urocanase, glycine cleavage enzyme, N^{5,10}-methylene-tetrahydrofolate reductase, serine-hydroxymethylase, formyltetrahydrofolate synthetase, and formiminotransferase.

MATERIALS AND METHODS

Histidase activity of the rat liver was estimated by Tabor and Mehler's method (1955): 200 mg of the liver specimens were homogenized with 4 ml of 0.1 M sodium pyrophosphate buffer of pH 9.2, and the homogenate was centrifuged at $600 \times g$ for 10 min, then 0.4 ml of the supernatant was used for the assay.

Urocanase activity of the rat liver was estimated by Tabor and Mehler's method (1955): 200 mg of the liver specimens were homogenized with 5 ml of saline, and the homogenate was centrifuged at $600 \times g$ for 10 min, then 0.5 ml of the supernatant was used for the assay.

N^{5,10}-methylenetetrahydrofolate reductase activity of the rat liver was estimated by Kutzbach *et al.*'s method (1967) and the detailed procedures were the same as described in a previous paper of ours (Narisawa *et al.* 1968) except that 0.41 μ moles of ¹⁴C-methyl-labeled-N⁵-methyl-DL-tetrahydrofolic acid (specific activity of 2,997 cpm per $m\mu$ mole) was used.

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TABLE 1. *In vitro* study of inhibitory effect of diphenylhydantoin upon glycine-cleavage tetrahydrofolate reductase, formiminotransferase,

Enzyme activity Amount of diphenyl- hydantoin added to the reaction mixture (per ml)	Glycine-cleavage enzyme (cpm of $^{14}\text{CO}_2$ from glycine- $1\text{-}^{14}\text{C}$ per 10 mg protein per hour)	$\text{N}^5,^{10}$ methylene- tetrahydrofolate reductase (cpm of ^{14}C - formaldehyde per mg protein per hour)	Serine-hydroxy- methylase (m μ moles of $\text{N}^5,^{10}$ - methenyltetrahydro- folate per mg protein per hour)
0	3,053	20,243	1,248
50 γ	3,063	20,643	1,264
100 γ	3,165	20,178	1,440

The activities of formyltetrahydrofolate synthetase and serine hydroxymethylase activity of the rat liver were estimated by Bertino *et al.*'s method (1962) and detailed procedures were the same as described in a previous paper of ours (Arakawa *et al.* 1967) except that 1 g of the liver specimen was homogenized with 7 ml of 0.05 M potassium phosphate buffer of pH 7.5 and the homogenate was centrifuged at $600 \times g$ for 10 min, then the supernatant was diluted with iced water up to 1:4, 0.2 ml of which being used for assay.

The activity of the glycine cleavage enzyme of the rat liver was estimated in the same way as described in a previous paper of ours (Tada *et al.* 1969).

The formiminotransferase activity of rat livers was estimated by Tabor and Wyngarden's method (1959).

A stock solution of diphenylhydantoin was prepared by dissolving 50 mg of sodium diphenylhydantoin in 1.0 ml of a solvent containing 40% propylene glycol and 10.5% ethanol. The stock solution was diluted with water and this was used for the experiment.

RESULTS AND DISCUSSION

The results were shown in Table 1, revealing that diphenylhydantoin, at a concentration of 50 γ per ml of the reaction mixture, had no inhibitory action upon glycine cleavage enzyme, $\text{N}^5,^{10}$ -methylenetetrahydrofolate reductase, serine-hydroxymethylase, formyltetrahydrofolate synthetase, formiminotransferase, histidase and urocanase of the rat liver.

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enzyme, serine-hydroxymethylase, formyltetrahydrofolate synthetase, N⁵,¹⁰-methylene-histidase and urocanase of rat liver

Formyltetrahydrofolate synthetase (mμ moles of N ⁵ , ¹⁰ -methenyltetrahydrofolate per mg protein per hour)	Formiminotransferase (mμ moles of N ⁵ , ¹⁰ -methenyltetrahydrofolate per min per mg wet weight)	Histidase (μ moles of urocanate formed per g wet weight per hour)	Urocanase (μ moles of urocanate disappeared per g wet weight per hour)
720	0.520	39.0	9.82
704	0.510	38.7	9.42
704		37.8	9.66

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