Note

Studies on the Metabolism and Toxicity of Dinitrotoluenes

—Changes of excretion, distribution and metabolism of

^3H -2,4-dinitrotoluene (^3H -2,4-DNT) in rats—†

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1. Introduction

In our previous studies^{1),2)}, the present authors have demonstrated the features of elimination and distribution of the radioactivity in rats after single oral administration of ^3H -2,4-DNT and have confirmed that the extent of radioactivity of the fecal excretion is much greater than that of urinary excretion, moreover, liver, skin and adipose tissue tend to accumulate radioactive unchanged 2,4-DNT and/or its metabolites. On the basis of the results, we have subsequently studied the elimination and distribution of radioactivity of ^3H -2,4-DNT in rats fed with a 0.5% 2,4-DNT-diet for long periods in order to understand the mechanism of toxic actions of 2,4-DNT in detail.

In this paper we report the change in the urinary and fecal excretions and distribution of ^3H -2,4-DNT in rats ingesting 0.5% 2,4-DNT-diet for four months. The influence of dietary 2,4-DNT on the reductase activity is also described.

2. Experimental

Animal feeding and administration of ^3H -2,

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4-DNT: Animals (7-week-old, male rat of Wistar strain) were divided into two groups consisting of 10 and 10 animals. Standard diet was given to 10 rats (control group) and the standard diet containing 0.5% 2,4-DNT was given 10 rats (2,4-DNT treated group). After feeding for four months, ^3H -2,4-DNT was given orally into 5 rats of control group and 5 rats of 2,4-DNT treated group in a dose of 1.76 MBq/kg (47.6 $\mu\text{Ci/kg}$ (50 mg/kg)). The urine and feces were collected separately every day during the following seven days.

Excretion studies: Collected feces samples were solubilized by the method previously described¹⁾ and then the radioactivity was determined by liquid scintillation counting. The urine samples were applied to the determination of the radioactivity.

Distribution studies: Control and 2,4-DNT treated rats were sacrificed by decapitation seven days after administration of ^3H -2,4-DNT. The whole organs excised were homogenized and was solubilized by the method previously described¹⁾, and then were applied to the determination of the radioactivity.

Metabolism studies: 9000 g Supernatant, enzyme solution, were prepared separately from livers of 5 rats and 5 rats of control and 2,4-DNT treated groups according to the conventional method. The incubation medium (11.1 ml) contained 5.0 ml of 0.1 M phosphate buffer

(pH 7.4), 1.0 ml of NADPH (2.5 $\mu\text{mol/ml}$), 2.0 ml of MgCl_2 (2.0 $\mu\text{mol/ml}$), 3.0 ml of 9000 g supernatant solution and 1.1 μmol of ^3H -2,4-DNT in 0.1 ml of ethanol solution. The incubation was carried out at 37° for 20 min anaerobically in Thunberg vessel under an N_2 atmosphere. The sample preparation for assay of reductase activity was carried out according to the previously method³⁾, which was carried out by the preparative thin-layer chromatography utilizing 2-amino-4-nitrotoluene (2A4NT) and 4-amino-2-nitrotoluene (4A2NT), reduction metabolites of 2,4-DNT. The samples obtained were submitted to liquid scintillation counter for the determination of the radioactive metabolites.

3. Results and Discussion

Excretion study—The cumulative excretion of the radioactivity after administration of ^3H -2,4-DNT in the rats is shown in Fig. 1. The features of excretion of the radioactivity in the urine and feces in the control rats are similar to our previous findings^{1),2)} in rats after single oral administration of ^3H -2,4-DNT. Accordingly, large amount of ^3H -2,4-DNT administered was excreted in the feces rather than in the

Fig. 1 Cumulative excretion of radioactivity after administration of ^3H -2, 4-DNT in 2, 4-DNT treated and control rats. Each point represents the mean of five rats.

urine in the control rats. In the 2,4-DNT treated rats, however, the radioactivity level between the urinary and fecal excretions was reversed distinctly, eventually, the amount of radioactivity excreted into the urine was much greater than that excreted into the feces.

Distribution study—Fig. 2 shows the tissue distribution of the radioactivity seven days after administration of ^3H -2,4-DNT. A striking observation is the high amount of radioactivity in the livers of control and 2,4-DNT treated rats. The high level of radioactivity in the skin and adipose tissue is also observed commonly in the both of control and 2,4-DNT treated rats. Though the radioactivity level of each organ in the 2,4-DNT treated rats is relatively lower than that in the control rats, it seems to suggest that the organs of 2,4-DNT treated rats are saturated to some extent by ingesting 0.5% 2,4-DNT-diet for four months. In addition, the relative tissue distribution of radioactivity in the control rats is similar to our previous finding¹⁾ in rats after single oral administration of ^3H -2,4-DNT.

Fig. 2 Distribution of radioactivity in the tissues one week after administration of ^3H -2, 4-DNT in 2,4-DNT treated and control rats. Each value represents the mean of five rats.

From these results it is confirmed, as expected in our previous finding¹⁾, that the liver, skin and adipose tissue tend to accumulate the unchanged 2,4-DNT and/or its metabolites.

Metabolism study—The amounts of radioactive metabolites and unchanged ^3H -2,4-DNT

Table 1 Determination of radioactivities corresponding to the unchanged 2,4-dinitrotoluene and two monoaminonitrotoluenes formed by 9000 g supernatant of rat liver

Animals (No.)	2,4-DNT (%)	2A4NT(%)	4A2NT(%)
Control (5)	27.31±0.09	22.10±0.26	48.59±1.07
2,4-DNT treated (5)	56.14± 0.51***	12.48± 1.81***	30.05± 2.19***

Each value is the mean of three experiments with the standard deviation. Significant difference is at $p < 0.01$ (***) as compared with control value.

are listed in Table 1. The amounts of ^3H -2A-4NT and ^3H -4A2NT, reductive metabolites of ^3H -2,4-DNT, and unchanged form in control rats are about 22 and 50, and 27 per cent, respectively. This formation ratio is nearly identical with our previous finding³⁾ which was obtained from 9000 g supernatant of rat liver incubated with nonlabelled 2,4-DNT. While the amounts of the two reductive metabolites formed by 9000 g supernatant of liver of 2,4-DNT treated rats markedly decreased compared

with those of control rats.

From these results it is evident that the reductase activity in the liver of 2,4-DNT treated rats is much lower than that in the liver of control rats. This finding suggests that the reductase activity of 2,4-DNT in rat liver is markedly decreased by ingesting 0.5% 2,4-DNT-diet for four months.

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