Studies on the Metabolism and Toxicity of Dinitrotoluenes

—On the Absorption and Excretion of Tritium-labelled 2,4-Dinitrotoluene (^3H -2,4-DNT) in the Rat—

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In order to investigate the detailed absorption and excretion of 2,4-DNT in the rat, after the single oral administration of ^3H -2,4-DNT, the time-course of radioactivity level in blood, liver and digestive organ contents, and of biliary, faecal and urinary excretion were observed.

Radioactivity level in blood reaches a peak at 6 hr after the administration and its half-life was about 22 hr. Maximum level of radioactivity in the liver was observed at 6 hr after the administration as well as in the blood. In contrast with the radioactivity levels in blood and liver, amount of radioactivity in small intestine content is markedly decreased for 6 hr after administration.

In the biliary excretion, about 10 per cent of the radioactivity administered was excreted in bile within 24 hr. The excretion rate is rising steadily from 6 hr after administration and its peak time was 9–10 hr. In the faecal excretion, the most of the excretion radioactivity was concentrated on 6–9 hr faeces as reflected with the biliary excretion pattern. On the other hand, the most of the urinary excretion radioactivity was concentrated on 0–6 hr urine.

1. Introduction

In an earlier paper¹⁾ dealing with the excretion and distribution studies of ^3H -2,4-DNT in the rat, it was shown that the most of the excretion radioactivity was concentrated on the first day-faeces and the first day-urine, and that relatively high level of radioactivity was maintained in liver together with adipose tissue and skin at seven days after administration. Accordingly, the detailed examination on absorption and excretion of ^3H -2,4-DNT will give the significant information regarding studies on metabolism and toxicity of 2,4-DNT.

The present report is concerned with the time-course of the radioactivity level in blood, liver and digestive organ contents after single oral administration of ^3H -2,4-DNT in the rat. In addition, the time-course of biliary, faecal and urinary excretion within 24 hr after administration of ^3H -2,4-DNT is also described.

2. Experimental

Absorption and distribution studies: Twenty

four male rats (Wistar strain, 200 g) were given p. o. at a dose of $47.6 \mu\text{Ci/kg}$ (22.2 mg/kg) with a salad oil solution of ^3H -2,4-DNT. Blood sample (0.5 ml) was taken from jugular vein at certain time intervals. After collecting the blood samples, rats were sacrificed by anesthetization and then livers, stomachs, small and large intestine contents were removed. Radioactivities of these samples were determined by liquid scintillation counting.

Biological half-life: Disappearance rate of radioactivity from blood was calculated from blood sample, and expressed as the biological half-life ($t_{1/2}$) according to standard "decay" equations.²⁾

$$1) \frac{N_t}{N_0} = e^{-\lambda t}, \quad 2) t_{1/2} = \frac{\ln 2}{\lambda}$$

where N_t = concentration of radioactivity after " t " hr, N_0 = initial concentration of radioactivity ($t=0$), λ = "decay" constant, t = time (hr), and e = base of natural logarithm ($\ln$).

Urinary, faecal and biliary excretion studies: Three male rats were administered p. o. at a dose of $47.6 \mu\text{Ci/kg}$ (22.2 mg/kg) with a salad

oil solution of ^3H -2,4-DNT. Urine and faeces were collected for 0-3 hr, 3-6 hr, 6-9 hr and 9-24 hr. For the biliary excretion study, after three male rats were anesthetized with ether and bile duct was cannulated PE-5 tube (0.8 mm)³¹, was administered p. o. at a dose of 46.2 $\mu\text{Ci/kg}$ (21.6 mg/kg) with a salad oil solution of ^3H -2,4-DNT. Bile samples were collected hourly for 24 hr.

Sample preparation for scintillation counting: An aliquot of urine (0.2 ml) and bile (0.1-0.6 ml) were dissolved in 10 ml of Bray's solution and then was counted directly. About 50 mg of homogenated liver and stomach, small and large intestine contents and about 10 mg of powdered faeces were dissolved in 2 ml of Scintillamine-OH solution at 50° for over night and then was counted in 10 ml of 30% ethanol-toluene scintillator. The blood sample (0.5 ml) was dissolved in a mixture solvent of Protosol-ethanol (1:2) at 60° for 1 hr by shaking and then was cooled in room temperature, and was decolorized with 30% hydrogen peroxide, and was incubated at 60° for 30 min^{41,32}. The sample obtained was counted in 10 ml of Biofluor solution³³. The instrument used for determination of radioactivity was a Aloka Model LSC-671 Liquid Scintillation Counter.

3. Results and discussion

Absorption and distribution studies—The radioactivity level in blood at specified time intervals after oral administration of ^3H -2,4-DNT is shown in Fig. 1. Radioactivity level in the blood reaches a peak at 6 hr and gradually declines over the first 9 hr. At 136 hr after administration the radioactivity level in blood is approximately 33 per cent compared with the level at 6 hr.

The result in Fig. 2 indicates a biological half-life of the radioactivity of about 22 hr in the blood, and this value is quite long comparing with the behaviour of other drugs such as phenylbutazone (about 6 hr)⁷ and antipyrine (about 2.3 hr)³⁴ in the rat. In the liver, radioactivity level reaches a peak at 6 hr after administration (Fig. 3) and its peak time paralleled to that of blood. The time-course of the radioactivity level in contents of digestive organs is shown in Fig. 4. Radioactivity levels in stomach and small intes-

Fig. 1 Time-course of radioactivity level in the blood of rats after oral administration of ^3H -2,4-DNT.

Each point is means of three rats with standard deviation and is expressed as the radioactivity per milliliter of blood at the different times after administration of ^3H -2,4-DNT (22.2 mg/kg, 47.6 $\mu\text{Ci/kg}$ p.o.).

Fig. 2 Biological half-life of ^3H -2,4-DNT in the rat.

Each point represents logarithm of concentration of ^3H -2,4-DNT per milliliter blood at the different times and each value of logarithm plots with graduation of equal intervals (Vertical axis). Biological half-life calculated by standard "decay" equations (Experimental).

tine contents are rapidly declined at 6 hr after oral administration of ^3H -2,4-DNT.

These results indicate that the peak time of absorption from small intestine of radioactivity administered orally is approximately 6 hr. The increase of radioactivity levels in small and large intestine contents at 9 hr depends on biliary excretion of certain radioactivity as described below.

Biliary, faecal and urinary excretion studies

Fig. 3 Time-course of radioactivity level in the liver of rats after oral administration of ^3H -2,4-DNT. Each point is means of three rats with standard deviation.

Fig. 4 Time-course of radioactivity level in the stomach, small and large intestine contents of rats after oral administration of ^3H -2,4-DNT.

Each point is means of three rats with standard deviation.

—Radioactivity level in the bile at different times after oral administration of ^3H -2,4-DNT is shown in Fig. 5. The individual excretion curve in Fig. 5 is revealed a similar pattern among three rats, the radioactivity level reaches a maximum at 9-10 hr after oral administration. About 10 per cent of the orally administered radioactivity is excreted in the bile within 24 hr.

A considerable amount of radioactivity is excreted in the 6-9 hr faeces (Fig. 6). Based on the results of biliary excretion, it appears that a peak time of faecal excretion depends on

Fig. 5 Time-course of radioactivity levels in the bile of three rats after oral administration of ^3H -2,4-DNT (22.0 mg/kg, 46.2 μCi /kg).

Fig. 6 Time-course of faecal excretion of radioactivity after ^3H -2,4-DNT administration.

Each value represents the mean $\pm$ standard deviation from three rats and expressed as the radioactivity in faeces at the different times after administration of ^3H -2,4-DNT (22.2 mg/kg, 47.6 μCi /kg).

the biliary excretion pattern.

As shown in Fig. 7, the excretion rate of urinary radioactivity is more rapid than that of faecal excretion and about 60 per cent of the radioactivity is excreted within the first 6 hr. This excretion pattern parallel to the time-course

Fig. 7 Time-course of urinary excretion of radioactivity after ^3H -2, 4-DNT administration.

Each value represents the mean $\pm$ standard deviation from three rats and is expressed as the radioactivity in urine at the different times after administration of ^3H -2,4-DNT (22.2 mg/kg, 47.6 $\mu\text{Ci/kg}$).

of the radioactivity level in blood.

Based on the present results, it is suggested that the absorption rate from the digestive organs of ^3H -2,4-DNT is relatively slow and that a half-life in blood is longer than other drugs such as phenylbutazone⁷⁾ and antipyrine.⁸⁾ Furthermore, it is also suggested that the most of the faecal excretion radioactivity is originated

in the biliary excretion of the radioactivities.

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要 旨

ジニトロトルエン類の代謝と毒性に関する研究
——ラットにおける ^3H 標識 2,4-ジニトロトルエン
(^3H -2,4-DNT) の吸収および排泄について——

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ラットにおける2,4-ジニトロトルエンの詳細な吸収および排泄について研究するために, ^3H -標識2,4-ジニトロトルエン1回経口投与後の血中, 肝中および消化管内容物中の放射能の経時変化ならびに胆汁, 糞および尿中排泄放射能の経時変化について観察した。

血中放射能は投与後6時間で最大となりその半減期は約22時間であった。肝中の放射能も血中の場合と同様に投与後6時間で最大になった。これらの結果とは対照的に小腸内容物中の放射能は投与後6時間で著しく減少していた。一方, 胆汁中には投与後24時間以内に約10%の放射能が排泄されたが排泄速度は投与後6時間目から上昇し9~10時間目で最大になった。この胆汁排泄様式を反映して糞中排泄放射能の大半は6~9時間目に集中していた。尿中放射能の大半は糞中排泄の場合とは逆に, 投与後6時間以内に集中していた。