Effect of Several Xenobiotics on the Activities of Enzymes Affecting Ascorbic Acid Synthesis in Rats

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Summary The dietary addition of several xenobiotics, such as PCB, DDT, aminopyrine, chloretone, BHT and BHA, caused significant increases in the ascorbic acid in urine and liver of rats. The administration of all types of xenobiotics used in the present experiments increased the activity of hepatic UDP-glucose dehydrogenase (1.3~2.8-fold), and the administration of PCB, DDT, BHT or BHA significantly increased the activity of hepatic UDP-glucuronyl transferase (2.2~13.1-fold). The activity of β-glucuronidase was slightly increased with feeding of PCB, DDT, chloretone or aminopyrine. However, the activity of hepatic UDP-glucuronic acid pyrophosphatase, the conversion of D-glucuronic acid or D-glucuronolactone into L-ascorbic acid and the activity of hepatic L-gulonolactone oxidase did not increase with the administration of PCB or DDT. It is suggested that the increases in the activities of UDP-glucose dehydrogenase and UDP-glucuronol transferase would have a major role in the stimulation of ascorbic acid synthesis in xenobiotic treated rats.

Key Words xenobiotics, PCB, ascorbic acid synthesis, glucuronic acid pathway

Many xenobiotics, such as PCB2, DDT and various low molecular weight fat-soluble chemicals, influence many metabolic states in vivo. For example, the following changes were generally observed with xenobiotic feeding in rats: an increase in the activity of hepatic drug-metabolizing enzymes (1-4), lipid accumulation in liver (5), a rise in plasma level of cholesterol (6-9) and increases of ascorbic acid in tissues and urine (10-21). Furthermore, the administration of xenobiotics may affect the requirements of certain nutrients.

We have an interest in the marked increases in urinary excretion of ascorbic

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2 Abbreviations: PCB, polychlorinated biphenyls; DDT, 1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane.
acid and tissue levels of ascorbic acid, which is caused by the administration of various types of xenobiotics. In rats, ascorbic acid is synthesized in the liver via the glucuronic acid pathway (Fig. 1). Conny et al. (15) and Horio and Yoshida (21) reported that this phenomenon was caused by the stimulation of ascorbic acid biosynthesis in liver. Burns et al. (12) clearly demonstrated that increased urinary ascorbic acid caused by barbital and by chloretone was the result of enhanced conversion of hexose to the vitamin and its precursors.

However, for the glucuronic acid pathway, the rate limiting step of biosynthesis of L-ascorbic acid has not been elucidated. Conny et al. (15) reported a two-fold increase in the activity of hepatic uridine diphosphate glucose dehydrogenase (UDP-glucose dehydrogenase) in rats following chloretone pretreatment. Many studies have since been reported on the effects of various xenobiotics on the activities of hepatic UDP-glucose dehydrogenase, uridine diphosphate glucuronyl transferase (UDP-glucuronyl transferase), uridine diphosphate glucuronic acid pyrophosphatase (UDP-glucuronic acid pyrophosphatase) (22) and L-gulonolactone oxidase (23). Notten et al. (24) suggested that stimulation of the glucuronic acid pathway by phenobarbital was based on an enhanced availability of UDP-glucose, and measured liver levels of UDP-glucose and UDP-glucuronic acid in rats treated with various compounds (27). Few studies have been reported concerning the overall changes in the enzyme activities in the glucuronic acid pathway in rats administered xenobiotics.

It was the purpose of the present study to ascertain which enzyme activity in the glucuronic acid pathway was affected by the administration of several xenobiotics, including PCB, DDT, aminopyrine, chloretone, butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA).

Fig. 1. Glucuronic acid pathway. (1) UDP-glucose dehydrogenase, (2) UDP-glucuronyl transferase, (3) UDP-glucuronic acid pyrophosphatase, (4) β-glucuronidase, (5) conversion of D-glucuronic acid to L-ascorbic acid, (6) conversion of D-glucuronolactone to L-ascorbic acid, (7) L-gulonolactone oxidase.
MATERIALS AND METHODS

*Animals and diets.* Young male Wistar rats weighing about 110–120 g were used in all experiments. Rats were fed a commercial stock diet\(^3\) for 2 days and then fed a 30% casein diet (basal diet) for 2 days before feeding the experimental diets. The experimental diets were the basal diets with or without the addition of xenobiotics. The composition of basal diet is shown in Table 1. Room temperature was kept at 24°C with 12 h cycle of light (from 08.00 to 20.00 o’clock) and dark. All rats were individually housed, provided feed and water *ad libitum*. The number of rats in each group was five or six.

*Experiment 1–3.* Rats of several groups fed a different test diet for 14 days: the basal diet (control group) and the basal diet supplemented with one of the following compounds; 200 ppm PCB\(^4\) (200 ppm PCB group), 500 ppm DDT (500 ppm DDT group) or 1,000 ppm aminopyrine (1,000 ppm aminopyrine group). On day 10 to 11, urine was collected into 50 ml of deionized water and used for the determination of glucuronic acid. Furthermore, on day 11 to 12, urine was collected into 50 ml of 5% metaphosphoric acid solution and used for the determination of ascorbic acid.

*Experiment 4.* Rats of other groups fed a different test diet for 10 days: the basal diet (control group) and the basal diet supplemented with one of the following

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### Table 1. Composition of the basal diet.

| Constituents                      | %       |
|----------------------------------|---------|
| Casein                           | 30      |
| Salt mixture\(^a\)               | 5       |
| Vitamin mixture\(^b\)            | 0.5     |
| Choline chloride                 | 0.2     |
| Corn oil                         | 5       |
| Cellulose powder                 | 4       |
| Starch : Sucrose (2 : 1)         | 55.3    |
| Retinyl palmitate                | 6.67 mg/kg |
| Ergocalciferol                   | 100 μg/kg |
| DL-α-Tocopheryl acetate          | 100 mg/kg |
| Se(Na\(_2\)SeO\(_3\))            | 0.1 mg/kg |

\(^a\) 5% of the salt mixture in the diet provides the following elements in mg/kg diet: Ca, 5,920; P, 3,940; K, 4,930; Na, 4,930; Cl, 7,600; Mg, 490; Fe, 49; Cu, 19; Mn, 19.5; Zn, 4; I, 0.19; Mo, 0.05; Se, 0.025 (36). \(^b\) 0.5% of the vitamin mixture in the diet provides the following vitamins in mg/kg diet: thiamine, 10; riboflavin, 10; nicotinic acid, 50; Ca-pantothenate, 40; pyridoxine-HCl, 5; folic acid, 0.4; menadione, 1; biotin, 0.2; vitamin B\(_12\), 0.04; inositol, 200; ascorbic acid was not contained (36).

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\(^3\) Commercial stock diet CE 2 from Japan Clea Co., Ltd., Tokyo, Japan.

\(^4\) Aroclor 1248, Mitsubishi Monsant Co., Ltd., Tokyo.
compounds; 2,000 ppm BHT (2,000 ppm BHT group), 3,000 ppm BHA (3,000 ppm BHA group) or 3,000 ppm chloretone (3,000 ppm chloretone group).

Experiment 5. Rats of six groups fed a different test diet for 14 days: the basal diet (control group) and the basal diet supplemented with one of the following compounds; 500 ppm DDT (500 ppm DDT group), 3,000 ppm chloretone (3,000 ppm chloretone group), 2,000 ppm BHT (2,000 ppm BHT group), 3,000 ppm BHA (3,000 ppm BHA group) or 1,000 ppm aminopyrine (1,000 ppm aminopyrine group).

We repeated the same experiments, because we couldn't measure the activities of several enzymes at the same time. At the end of the experimental period, rats were anesthetized with ether and killed at 13.00 o'clock within a 120 min period.

Analytical methods

Enzyme assays. UDP-glucose dehydrogenase activity was measured by the rate of NAD⁺ reduction spectrophotometrically at 340 nm according to Strominger et al. (25). For the determination of UDP-glucuronol transferase activity, liver homogenates (25% w/v) were prepared in 0.25 M sucrose solution containing 0.05 M Tris-HCl (pH 7.4) and centrifuged for 15 min at 10,000 × g (2°C). The supernatants were treated with Triton X-100 (0.25%, final concentration). The activity was measured according to the method of Henderson (26). UDP-glucuronic acid pyrophosphatase was assayed by the procedure of Ginsburg et al. (27), with some modifications. To determine the remaining UDP-glucuronic acid after stopping the enzymatic reaction, o-aminophenol was adopted as the acceptor and o-aminophenyl β-D-glucuronide was formed by the incubation with guinea-pig liver microsomes. The absorbancy of o-aminophenyl β-D-glucuronide was measured at 535 nm. β-Glucuronidase of liver was assayed using phenolphthalein β-D-glucuronide as substrate (28). This enzyme was located both in microsome and in lysosome. The crude liver homogenate (3.0% w/v) was used for the determination of total activity, and this homogenate was centrifuged for 15 min at 10,000 × g (2°C) to remove lysosome, mitochondria and nuclear. The postmitochondrial supernatant was used for the determination of activity in microsome. Biosynthesis of ascorbic acid from D-glucuronic acid or D-glucuronolactone was determined as described by Hassan and Lehninger (29). These pathway included some enzymatic reaction. The activity of hepatic L-gulonolactone oxidase was measured by the method of Chatterjee et al. (30). The measurement of the activities of all enzymes were carried out immediately after killing rats.

Measurements of ascorbic acid and glucuronic acid. The remaining liver, kidney and spleen were frozen with liquid nitrogen and then stored at −20°C until analyzed. The stored tissues were homogenized with ice-cold 5% metaphosphoric acid and centrifuged for 10 min at 3,000 rpm. Ascorbic acid in the supernatant was measured by the dinitrophenylhydrazine method (31). Ascorbic acid in collected urine was measured similarly. Glucuronic acid (free and conjugated) in urine was measured by Dische's carbazole reaction (32), modified by Yuki and Fishman (33).
Table 2. Effects of dietary addition of xenobiotics on body weight gain, liver weight and liver level of ascorbic acid.

|          | Initial body wt. (g) | Body wt. gain for 14 days or 10 days (g) | Liver wt. (g/100 g body wt.) | Liver level of ascorbic acid (µg/g) | Spleen level of ascorbic acid | Kidney level of ascorbic acid |
|----------|----------------------|------------------------------------------|--------------------------------|-------------------------------------|-----------------------------|-----------------------------|
| **Experiment 1** |                      |                                          |                                |                                     |                             |                             |
| Control (6) | 126.6 ± 1.7          | 104.6 ± 6.3                              | 4.53 ± 0.18<sup>a,3</sup>    | 244 ± 11<sup>a</sup>               |                             |                             |
| 200 ppm PCB (6) | 127.3 ± 1.2          | 89.7 ± 5.6                               | 6.69 ± 0.20<sup>b</sup>      | 559 ± 66<sup>b</sup>                |                             |                             |
| 500 ppm DDT (6) | 124.0 ± 1.2          | 105.5 ± 4.8                              | 5.91 ± 0.20<sup>b</sup>      | 531 ± 20<sup>b</sup>                |                             |                             |
| 1,000 ppm aminopyrine (6) | 122.4 ± 0.7           | 106.5 ± 4.0                              | 5.10 ± 0.06<sup>a</sup>      | 453 ± 30<sup>b</sup>                |                             |                             |
| **Experiment 2** |                      |                                          |                                |                                     |                             |                             |
| Control (6) | 122.0 ± 3.0          | 61.8 ± 4.0                               | 4.43 ± 0.07<sup>a</sup>      | 203 ± 19<sup>a</sup>                | 321 ± 11<sup>a</sup>       | 81 ± 3<sup>a</sup>          |
| 200 ppm PCB (6) | 122.0 ± 2.0          | 64.2 ± 2.2                               | 6.15 ± 0.12<sup>b</sup>      | 510 ± 69<sup>b</sup>                | 447 ± 13<sup>b</sup>       | 238 ± 13<sup>b</sup>        |
| 500 ppm DDT (6) | 122.0 ± 2.0          | 63.7 ± 3.9                               | 5.81 ± 0.25<sup>b</sup>      | 552 ± 83<sup>b</sup>                | 456 ± 12<sup>b</sup>       | 243 ± 5<sup>b</sup>         |
| **Experiment 3** |                      |                                          |                                |                                     |                             |                             |
| Control (5) | 117.8 ± 1.7          | 71.0 ± 2.5                               | 4.53 ± 0.05<sup>a</sup>      | 288 ± 14<sup>a</sup>                |                             |                             |
| 200 ppm PCB (5) | 117.2 ± 1.5          | 76.4 ± 3.6                               | 6.42 ± 0.12<sup>b</sup>      | 639 ± 27<sup>b</sup>                |                             |                             |
| **Experiment 4** |                      |                                          |                                |                                     |                             |                             |
| Control (5) | 107.8 ± 1.8          | 45.5 ± 1.8<sup>ab</sup>                  | 4.71 ± 0.09<sup>a</sup>      | 309 ± 11<sup>a</sup>                |                             |                             |
| 2,000 ppm BHT (5) | 106.3 ± 1.8          | 44.9 ± 3.3<sup>ab</sup>                  | 6.24 ± 0.08<sup>c</sup>      | 406 ± 13<sup>b</sup>                |                             |                             |
| 3,000 ppm BHA (5) | 106.4 ± 2.1          | 53.9 ± 1.6<sup>b</sup>                   | 5.68 ± 0.14<sup>b</sup>      | 343 ± 13<sup>a</sup>                |                             |                             |
| 3,000 ppm chloretone (5) | 106.8 ± 2.6          | 43.9 ± 3.1<sup>ab</sup>                  | 5.93 ± 0.13<sup>bc</sup>     | 794 ± 39<sup>c</sup>                |                             |                             |
| **Experiment 5** |                      |                                          |                                |                                     |                             |                             |
| Control (6) | 122.7 ± 1.7          | 76.8 ± 2.3                               | 5.01 ± 0.13<sup>a</sup>      | 307 ± 8<sup>a</sup>                 |                             |                             |
| 500 ppm DDT (6) | 120.7 ± 2.1          | 73.0 ± 3.0                               | 6.57 ± 0.11<sup>d</sup>      | 687 ± 17<sup>d</sup>                |                             |                             |
| 3,000 ppm chloretone (6) | 121.8 ± 2.1          | 79.6 ± 1.5                               | 5.96 ± 0.13<sup>e</sup>      | 799 ± 29<sup>e</sup>                |                             |                             |
| 2,000 ppm BHT (6) | 120.2 ± 2.2          | 70.3 ± 3.2                               | 6.44 ± 0.11<sup>d</sup>      | 418 ± 13<sup>b</sup>                |                             |                             |
| 3,000 ppm BHA (6) | 119.7 ± 2.0          | 73.0 ± 3.2                               | 5.50 ± 0.13<sup>b</sup>      | 333 ± 19<sup>ab</sup>               |                             |                             |
| 1,000 ppm aminopyrine (6) | 121.3 ± 1.9          | 69.7 ± 4.0                               | 5.24 ± 0.09<sup>ab</sup>     | 376 ± 30<sup>c</sup>                |                             |                             |

1 Experimental period was 14 days in Experiment 1–3, 5 or 10 days in Experiment 4, respectively. 2 Number of animals in each group is displayed within the parenthesis. 3 Mean ± SE. In each experiment, means within a column not followed by the same superscript letter are significantly different (p < 0.05).
Statistical significance of differences between values was analyzed by Duncan's multiple range test (34) or Student's $t$ test (37). In each experiment, the enzyme activities of rats administered xenobiotics were compared with the value of the control group, and statistical significance of differences was analyzed by Student's $t$ test.

RESULTS

Effects of various xenobiotics on body weight gain and liver weight

The administration of each xenobiotic had no effects on body weight gain (Table 2). The administration of PCB, DDT, BHT, BHA and chloretone caused marked enlargement of liver (Table 2).

Effects of various xenobiotics on levels of ascorbic acid in liver and urine and on urinary ascorbic acid

The levels of ascorbic acid in liver were significantly increased by feeding with all xenobiotics tested, except for BHA (Table 2). Moreover (data are not shown), other tissue (kidney, spleen) levels of ascorbic acid rose similarly with xenobiotic feeding. However, the increases in tissue levels of ascorbic acid with xenobiotic feeding were different among various tissues. In general, the concentrations of

| Urinary ascorbic acid (mg/day/100 g body wt.) | Urinary glucuronic acid |
|---------------------------------------------|------------------------|
|                                             | Conjugated (mg/day/100 g body wt.) | Free (mg/day/100 g body wt.) |
|---------------------------------------------|------------------------|
| Experiment 1                                |                        |
| Control (6)$^1$                             | 0.30 ± 0.06$^a, 2$     | —                      |
| 200 ppm PCB (6)                             | 11.31 ± 1.88$^b$       | —                      |
| 500 ppm DDT (6)                             | 9.70 ± 1.62$^b$        | —                      |
| 1,000 ppm aminopryrine (6)                  | 4.69 ± 0.61$^c$        | —                      |
| Experiment 2                                |                        |
| Control (6)                                 | 0.22 ± 0.04$^a$        | 4.39 ± 1.00            | 0.74 ± 0.33 |
| 200 ppm PCB (6)                             | 7.69 ± 0.48$^b$        | 7.88 ± 2.91            | 0.84 ± 0.28 |
| 500 ppm DDT (6)                             | 4.73 ± 0.64$^c$        | 8.40 ± 1.47            | 1.57 ± 0.37 |
| Experiment 3                                |                        |
| Control (5)                                 | 0.13 ± 0.01$^a$        | 2.06 ± 0.25$^a$        | 0.41 ± 0.27 |
| 200 ppm PCB (5)                             | 10.66 ± 0.55$^b$       | 4.59 ± 0.61$^b$        | 0.69 ± 0.43 |

$^1$ Number of animals in each group is displayed within the parenthesis. $^2$ Mean ± SE. In each experiment, means within a column not followed by the same superscript letter are significantly different ($p < 0.05$).
ascorbic acid were different in various tissues, in the following decreasing order: spleen, liver, kidney.

Urinary excretion of ascorbic acid increased markedly in the 200 ppm PCB group, 500 ppm DDT group and 1,000 ppm aminopyrine group (35–82-fold, 22–32-fold, 16-fold, respectively). In addition to urinary ascorbic acid, the

![Fig. 2. Effects of several xenobiotics on the activity of hepatic UDP-glucose dehydrogenase (Experiment 1 and 4). The enzyme activity is expressed as units per 100 g body weight. The bars indicate mean values and the vertical lines above the bars indicate the SE. Asterisks denote significant differences from the value of control group in each experiment (p<0.05). Cont., control group; PCB, 200 ppm PCB group; DDT, 500 ppm DDT group; AP, 1,000 ppm aminopyrine group; BHT, 2,000 ppm BTH group; BHA, 3,000 ppm BHA group; CHL, 3,000 ppm chloretone group.](image)

![Fig. 3. Effects of several xenobiotics on the activity of hepatic UDP-glucuronyl transferase (Experiment 2 and 5). The bars indicate mean values and the vertical lines above the bars indicate the SE. Asterisks denote significant differences from the value of control group in each experiment (p<0.05). Cont., control group; PCB, 200 ppm PCB group; DDT, 500 ppm DDT group; CHL, 3,000 ppm chloretone group; BHT, 2,000 ppm BTH group; BHA, 3,000 ppm BHA group; AP, 1,000 ppm aminopyrine group.](image)
administration of PCB, DDT or chloretone caused an increase in urinary excretion of glucuronic acid, especially in conjugated glucuronic acid (Table 3).

Effects of various xenobiotics on hepatic enzyme activities

1. UDP-glucose dehydrogenase. All enzyme activities were expressed as units per 100 g body weight. The ingestion of all types of xenobiotics significantly increased the activity of UDP-glucose dehydrogenase (PCB, 2.4-fold; DDT, 2.0-fold; aminopyrine, 1.3-fold; BHT, 2.5-fold; BHA, 2.8-fold; chloretone, 1.9-fold; respectively) (Fig. 2). We reported a part of this result in a previous paper (21).

2. UDP-glucuronyl transferase, UDP-glucuronic acid pyrophosphatase and β-

![Graph](https://example.com/graph.png)

Fig. 4. Effects of several xenobiotics on the activities of hepatic β-glucuronidase (Experiment 3 and 5) and UDP-glucuronic acid pyrophosphatase (Experiment 2). The bars indicate mean values and the vertical lines above the bars indicate the SE. Asterisks denote significant differences from the value of control group in each experiment (p<0.05). (A) β-glucuronidase, (B) UDP-glucuronic acid pyrophosphatase. Cont., control group; PCB, 200 ppm PCB group; DDT, 500 ppm DDT group; CHL, 3,000 ppm chloretone group; BHT, 2,000 ppm BHT group; BHA, 3,000 ppm BHA group; AP, 1,000 ppm aminopyrine group.

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glucuronidase. In PCB, DDT, BAT and BHA groups, marked increases in the activity of UDP-glucuronyl transferase were observed (to 13.1-fold, 3.4-fold, 2.7-fold, 2.2-fold, respectively) (Fig. 3). The administration of PCB or DDT did not affect hepatic UDP-glucuronic acid pyrophosphatase (Fig. 4). On hepatic β-glucuronidase activity, the administration of PCB, DDT, chloretone or aminopyrine caused slight increases in total activity. This enzyme was located in microsomes and lysosomes. The microsomal activity was increased only in PCB group.

3. Conversion of D-glucuronic acid or D-glucuronolactone to L-ascorbic acid. The activities of conversion from D-glucuronic acid or D-glucuronolactone to ascorbic acid in rats fed a basal diet, 200 ppm PCB diet or 500 ppm DDT diet are presented in Fig. 5. The xenobiotics used had no effect on the conversion of D-glucuronic acid. Moreover, no xenobiotics increased the amount of biosynthesis of ascorbic acid from D-glucuronolactone, the administration of DDT rather reduced the activity.

4. L-Gulonolactone oxidase. The activity of this enzyme was not affected by PCB or DDT compared to the control group (Fig. 5). Previously, we reported that the administration of aminopyrine had no effect on this enzyme activity (21).

5. Overall effect of dietary PCB on the activities of hepatic enzymes in ascorbic acid synthesizing pathway. On all enzyme activities, we measured the maximal
activity \((V_{\text{max}})\) under the appropriate condition. All enzyme activities in control group and 200 ppm PCB group are presented in Fig. 6. In both groups, the enzymes having high \(V_{\text{max}}\) were UDP-glucuronyl transferase and \(\beta\)-glucuronidase. The administration of PCB caused a 2.4-fold increase in UDP-glucose dehydrogenase, a 13.1-fold increase in UDP-glucuronyl transferase and a 1.3-fold increase in \(\beta\)-glucuronidase (total activity). Other enzyme activities were not significantly changed with PCB feeding.

The changes in enzyme activities with xenobiotic feeding are summarized in Table 4.

**DISCUSSION**

We used several xenobiotics, such as drugs, insecticides, antioxidants and others, in this study. These compounds had no effect on body weight gain at the levels in these experiments.
Table 4. Summary of the effects of several xenobiotics on the activities of enzymes relating ascorbic acid synthesis.

| Enzyme/Pathway                             | PCB | DDT | Aminopyrine | BHT | BHA | Chloretone |
|-------------------------------------------|-----|-----|-------------|-----|-----|------------|
| UDP-glucose dehydrogenase                 | +   | +   | +           | +   | +   | +          |
| UDP-glucuronyl transferase                | ++  | +++ | =           | +   | +   | =          |
| UDP-glucuronic acid pyrophosphatase       | =   | =   | N.D.        | N.D.| N.D.| N.D.      |
| β-Glucuronidase (total activity)          | +   | +   | +           | =   | =   | +          |
| D-Glucuronic acid → L-ascorbic acid       | =   | =   | N.D.        | N.D.| N.D.| N.D.      |
| D-Glucuronolactone → L-ascorbic acid      | =   | =   | N.D.        | N.D.| N.D.| N.D.      |
| L-Gulonolactone oxidase                   | =   | =   | =           | N.D.| N.D.| N.D.      |

=, not significantly changed; +, increased to 200% of the control level; ++, 200–300% of the control level; +++, more than 300% of the control level; −, significantly reduced; N.D., not determined. p < 0.05. 1 Data were published in previous report (21).

In general, the administration of xenobiotics induces the mixed function oxidase system accompanied by an enlargement of liver (1–4). The enlargement of liver is partially due to the lipid accumulation and the increase in the endoplasmic reticulum (5). The metabolic sequences leading to fatty liver is an interesting problem for future study.

The ingestion of xenobiotics caused marked increases in liver level of ascorbic acid and urinary excretion of ascorbic acid. It was found that maximum liver levels of ascorbic acid were 700–800 μg per g liver in rats fed diets containing xenobiotics. We reported in a previous paper (21) that the biosynthesis of ascorbic acid in rat was significantly increased with PCB feeding, and suggested that the rise in the biosynthesis of ascorbic acid led to increases in urinary and tissue ascorbic acid. In rats, ascorbic acid is synthesized via the glucuronic acid pathway in liver. However, no available information has been obtained concerning the rate-limiting step of the biosynthesis of ascorbic acid. Moreover, the glucuronic acid pathway is not a single pathway. Some steps of this pathway have two directions, for example, UDP-glucuronic acid, D-glucuronic acid and L-gulonic acid. The distribution of each direction have not been proved. We measured most of the activities of enzymes included in the pathway from UDP-glucose to L-ascorbic acid, and investigated the effects of dietary addition of xenobiotics, especially PCB and DDT, on the activity of each enzyme.
All types of xenobiotics used caused significant increases in the activity of UDP-glucose dehydrogenase (to 1.3-fold–2.8-fold). Good correlation was obtained between the liver level of ascorbic acid and the activity of hepatic UDP-glucose dehydrogenase (units per g liver) (21). Conney et al. (15) similarly demonstrated that the administration of chloretone to rat caused a two-fold increase in the activity of this enzyme. It is likely that the activity of hepatic UDP-glucose dehydrogenase has an important role in the increase of ascorbic acid synthesis.

The activity of UDP-glucuronyl transferase was greatly increased with the administration of PCB, DDT, BHT or BHA. Many xenobiotics are oxidized in vivo by hepatic drug-metabolizing enzymes, and subsequently conjugated with glucuronic acid or other compounds to be excreted into urine or bile. UDP-glucuronyl transferase catalyzes the conjugation of glucuronic acid to oxidized xenobiotics or endogenous acceptors. In addition, it is likely that this enzyme participates in the biosynthesis of ascorbic acid.

The administration of PCB, DDT, chloretone or aminopyrine caused a slight increase in the activity of hepatic β-glucuronidase. On measuring the activity, we used phenolphthalein-β-D-glucuronide as a substrate. Circulating β-glucuronides of some metabolites, such as steroids, in blood are divided into D-glucuronic acid and aglycones in target cells. On the other hand, β-glucuronidase probably participates in the synthesis of ascorbic acid.

On the other enzyme activities, the administration of xenobiotics did not have a significant effect.

Hollmann and Touster (22) reported that chloretone treatment increased the activities of UDP-glucose dehydrogenase and UDP-glucuronic acid pyrophosphatase and that aminopyrine treatment significantly increased the activity of UDP-glucuronyl transferase. In our results, the administration of aminopyrine slightly increased the activities of UDP-glucose dehydrogenase, UDP-glucuronyl transferase (not significantly), and β-glucuronidase.

Looking at the overall effects of PCB or DDT on enzyme activities in the ascorbic acid synthesizing pathway, the hepatic activities of UDP-glucose dehydrogenase and UDP-glucuronyl transferase were significantly increased. The $K_m$ value of UDP-glucose dehydrogenase we measured was 0.08 mM. The concentration of UDP-glucose was 0.418 mM in control group and 0.362 mM in PCB group (21), and these concentrations seemed to be higher than the $K_m$ of UDP-glucose dehydrogenase. It was reported by Notten and Henderson (17) that the concentration of liver UDP-glucuronic acid was increased with the administration of xenobiotics. On the pathway from the UDP-glucose to D-glucuronic acid, the flow of metabolites seemed to be mainly regulated by each enzyme activity, and not by the supply of each substrate. On the other hand, the activity of L-gulonolactone oxidase which catabolizes the last step of ascorbic acid synthesis was not affected with PCB or DDT feeding. The $K_m$ value of the enzyme we measured was 12.1 mM. Chatterjee (35) suggested that the liver level of L-gulonolactone was less than 0.05 mM. Therefore, in the pathway from D-glucuronic acid to L-ascorbic acid, the
flow of metabolites seemed to be regulated by the individual enzyme activities and the supply of each substrate.

It is suggested that the stimulation of ascorbic acid synthesis by the administration of xenobiotics is the result of increased activities of hepatic UDP-glucose dehydrogenase and UDP-glucuronol transferase. However, further studies are needed for a clearer conclusion.

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