EFFECT OF PCB (POLYCHLOROBIPHENYLS) ON L-ASCORBIC ACID, PYRIDOXAL PHOSPHATE AND RIBOFLAVIN CONTENTS IN VARIOUS ORGANS AND ON HEPATIC METABOLISM OF L-ASCORBIC ACID IN THE RAT

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Abstract—Effects of continuous oral administration of PCB (polychlorobiphenyls, 10-100 mg/kg/day, 4 weeks) on tissue levels of L-ascorbic acid (vitamin C), pyridoxal phosphate and riboflavin (vitamin B2) in various organs and on hepatic metabolism of L-ascorbic acid were examined in male Wistar rats weighing 150-250 g. Riboflavin contents in the liver, kidney, brain, heart and testis were not altered by PCB treatments, whereas the hepatic level of pyridoxal phosphate, a biologically active form of vitamin B6, was significantly reduced by PCB administration. Under the same experimental conditions, L-ascorbic acid contents in the liver, kidney, lung and testis showed a significant increase. Histochemical studies revealed that in the adrenal gland, increase of L-ascorbic acid was localized in the fasciculate and reticular zones of cortex, respectively. It was found that increase of L-ascorbic acid in the liver is caused predominantly by activation of biosynthesis at the steps of galactose to D-glucuronic acid and is not due to changes in the catabolic processes of L-ascorbic acid per se. Possible significance of these changes in tissue levels and/or metabolism of vitamins in the occurrence of PCB intoxication is briefly discussed.

It has been reported that PCB (polychlorobiphenyls) administered orally is deposited to a large extent in the liver and induces various pathological changes in the liver such as enlargement with fatty degeneration [1], proliferation of smooth surfaced endoplasmic reticulum [2, 3], enhancement of hepatic drug metabolism [1, 4-6], inhibition of activities of ATP-ase, glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase [1], and oxidative phosphorylation in hepatic mitochondria [1]. Concerning the effect of PCB on hepatic metabolism of vitamins, however, little information is available, although Villeluneve et al. [7] reported that hepatic vitamin A content in PCB-treated animals exhibited a significant decrease. It is well known that PCB intoxication induces peculiar skin lesions characterized by symptoms such as follicular accentuation, acneform eruption, pigmentation and hypersecretion of Meibomian glands [8-10]. These facts suggest that metabolism of other vitamins possibly involved in the maintenance of physiological functions of the skin may also be altered in these PCB-intoxicated animals.

We determined the effect of continuous administration of PCB on L-ascorbic acid (vitamin C), pyridoxal phosphate and riboflavin (vitamin B2) contents in various organs and on hepatic metabolism of L-ascorbic acid in the rat.
MATERIALS AND METHODS

**Animals:** Male Wistar rats weighing 150-250 g were fed for 4 weeks with commercial solid diets containing 10 and 100 mg/kg/day of PCB (Kanechlor). The mixture of equi-amounts of Kanechlor 300, 400, 500 and 600 was dissolved in acetone and required amounts of the PCB suspension were mixed with solid diets and dried. Control animals were provided solid diets pretreated with an equi-amount of acetone. Water was given ad libitum and all animals were kept under exactly the same nutritional and environmental conditions. At the end of 4 weeks, rats were decapitated and various organs were used for chemical and enzymatic assays (liver, kidney, brain, heart, lung, testis and adrenal gland) were removed.

**Chemical assays:** Riboflavin (vitamin B2) [11] and pyridoxal phosphate [12] were measured spectrofluorometrically, while the contents of L-ascorbic acid (vitamin C) [13] and protein [14] were assayed spectrophotometrically according to the previously described procedures, respectively.

**Measurements of the conversion of galactose to glucuronic acid:** The conversion of D-(1-14C) galactose to glucuronic acid in homogenates of the rat liver was determined by the method of Evans et al. [15]. The assay conditions used were as follows; Three ml of a 30% liver homogenate in 1.15% potassium chloride was added to a solution containing 10 mg ATP and 3 mg UDPG in 1 ml of 0.25 M phosphate buffer (pH 7.8), 0.1 ml of 0.25 M MgCl₂, and 0.2 ml of 1 M Tris buffer (pH 8.62). Two mg of D-(1-14C) galactose (S.A.; 0.027 mCi/mmmole) in 0.7 ml of water was then added to give a final volume of 5 ml and a pH of 7.8. The mixture was incubated in 30 ml beakers with shaking at 37°C for 30 min. After cooling in ice, 10 mg of NAD in 1.33 ml of 1 M Tris buffer (pH 8.62), 0.67 ml of 0.6 M nicotinamide, and 3 ml of fresh 30% liver homogenate were added to give a final volume of 10 ml and a pH of 8.6. The mixture was again incubated for 30 min and chilled. Following the addition of carrier D-glucuronic acid (300 mg), sufficient trichloroacetic acid solution was added to obtain a final concentration of 5% and the resulting mixture was centrifuged. The supernatant was placed on an Amberlite CG-4B anion exchange column in the acetate form and absorbed D-glucuronic acid was eluted with 2N formic acid. The radioactivity found in D-glucuronic acid fraction and total radioactivities recovered from the column were measured as previously described [16]. D-(1-14C)-galactose (S.A.; 59 mCi/mmmole) was obtained from the Radiochemical Center, Amersham, England.

The conversion rate of D-(1-14C)-galactose was calculated as follows:

\[
\text{Conversion rate} = \frac{\text{D-(1-14C)-glucuronic acid counts obtained}}{\text{total 14C counts recovered}} \times 100
\]

**Measurements of the formation of L-ascorbic acid from D-glucuronolactone:** The formation of L-ascorbic acid from D-glucuronolactone in rat liver homogenates was measured according to the procedures described by Mukherjee et al. [17]. The test system contained sodium phosphate buffer, pH 7.4 (20 mM), D-glucuronolactone (10 mM), 1 ml of a 20% fresh liver homogenate in 0.25 M sucrose and KCN (50 mM). The total volume was 5 ml, and the mixture was incubated at 37°C for 90 min. The reaction was terminated.
by the addition of 1 ml of 30% metaphosphoric acid solution. The precipitated protein was removed by filtration and the synthesized L-ascorbic acid was determined titrimetrically [18] with 2,6-dichlorophenol-indophenol reagent.

Measurements of the catabolism of L-ascorbic acid: The catabolism of L-ascorbic acid in rat liver homogenates was measured by the method of Mukherjee et al. [17]. The test system contained dehydroascorbic acid (10 μmoles, freshly prepared by Br₂ oxidation of ascorbic acid), 0.5 ml of a 20% liver homogenate in 0.25 M sucrose, GSH (0.3 μmole), Tris-maleate buffer, pH 6.8 (200 μmole), and MgCl₂ (20 μmole). The final volume was 3 ml. The reaction mixture was incubated at 37°C for 15 min and the reaction terminated by addition of 1 ml of 20% (w/v) metaphosphoric acid containing 2% (w/v) SnCl₂. The remaining dehydroascorbate was rapidly reduced with H₂S and filtered. After removal of excess H₂S in the filtrate by bubbling with a stream of CO₂, the 2,3-dioxogulonic acid formed was determined with 2,4-dinitrophenylhydrazine [13].

RESULTS

Effects of continuous oral administration of PCB on riboflavin content in various organs

Tissue levels of riboflavin (vitamin B₂) in liver, kidney, brain, heart, lung and testis following continuous administration of PCB for 4 weeks are shown in Table 1. No significant changes in the content of riboflavin were observed in these organs.

| Table 1. Effect of continuous oral administration of PCB on riboflavin (vitamin B₂) content in various organs of rats |
| --- |
| **Vitamin B₂ content (μg/g wet wt.) ± S.E.** |
| Control | 10 mg/kg/day | 100 mg/kg/day |
| Liver | 33.6 ± 2.2 | 36.5 ± 1.9 | 31.2 ± 2.7 |
| Kidney | 32.4 ± 1.7 | 34.4 ± 1.3 | 34.2 ± 1.4 |
| Brain | 3.3 ± 0.2 | 3.5 ± 0.2 | 3.3 ± 0.2 |
| Heart | 23.4 ± 1.3 | 22.6 ± 2.4 | 19.7 ± 1.6 |
| Lung | 4.3 ± 0.3 | 4.4 ± 0.3 | 4.3 ± 0.3 |
| Testis | 3.8 ± 0.2 | 4.0 ± 0.3 | 3.7 ± 0.2 |

Each value represents the mean of four rats ± standard error.

Administered orally for 4 weeks.

Table 2. Effect of continuous oral administration of PCB on pyridoxal phosphate (PAL-P) contents in liver and brain

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| --- |
| **Pyridoxal phosphate content (μg/g wet wt.) ± S.E.** |
| Control | 10 mg/kg/day | 100 mg/kg/day |
| Liver | 15.4 ± 0.7 | 9.2 ± 0.6** | 9.2 ± 0.7* |
| Brain | 4.4 ± 0.3 | 4.5 ± 0.3 | 4.5 ± 0.3 |

Each value represents the mean of four rats ± standard error.

Administered orally for 4 weeks.

* P < 0.05, ** P < 0.02, compared with the control value, respectively.
Effects of continuous oral administration of PCB on pyridoxal phosphate (PAL-P) contents in liver and brain

Table 2 shows the effect of continuous administration of PCB on the contents of PAL-P in the liver and brain.

The administration of 10 and 100 mg/kg/day of PCB for 4 weeks induced a significant decrease in the PAL-P content of the liver, whereas that in the brain was found to be essentially unchanged.

Effect of continuous oral administration of PCB on the tissue levels and metabolism of L-ascorbic acid

L-ascorbic acid contents in the liver, kidney, lung and testis showed a significant increase, whereas those in the brain and heart were not altered following the continuous administration of PCB (Table 3). The most significant increase was found in the liver and kidney. In the adrenal gland, a slight increase of L-ascorbic acid was noted in the group treated with 100 mg/kg/day of PCB for 4 weeks, but this increase was not statistically significant. Histochemical observations using the method of Eränkö [19], however, revealed that the L-ascorbic acid content in fasciculate zone of the cortex from animals treated with 10 and 100 mg/kg/day of PCB increased remarkably, while the content in reticular zone showed only a slight increase. In addition, L-ascorbic acid was not detected in glomerular zone of the cortex or in the medulla. These results suggested that increase in L-ascorbic acid contents in certain parts of the adrenal cortex following the PCB administration may also be detected if the microdistribution of this compound is examined.

Since the rat is known to be one species capable of synthesizing L-ascorbic acid [20], we examined the effects of continuous administration of PCB on the biosynthesis and catabolism of L-ascorbic acid in the liver in an attempt to clarify the cause of the increase of L-ascorbic acid in this organ. Regarding the synthesis of L-ascorbic acid, we studied the effect of PCB on the steps of D-galactose to D-glucuronic acid, and of D-glucuronolactone to L-ascorbic acid, respectively. In the first step D-galactose was chosen as the substrate,

Table 3. Effect of continuous oral administration of PCB on L-ascorbic acid content in various organs

| Organ          | Control        | 10 mg/kg/day | 100 mg/kg/day |
|----------------|----------------|--------------|---------------|
| Liver          | 176 ± 7        | 419 ± 36**   | 403 ± 53*     |
| Kidney         | 114 ± 7        | 310 ± 11**   | 363 ± 22*     |
| Brain          | 350 ± 30       | 363 ± 24     | 338 ± 23      |
| Heart          | 85 ± 5         | 102 ± 6      | 108 ± 9       |
| Lung           | 212 ± 8        | 313 ± 8***   | 319 ± 11***   |
| Testis         | 213 ± 6        | 277 ± 14***  | 276 ± 14***   |
| Adrenal gland  | 2970 ± 124     | 2770 ± 300   | 3610 ± 283    |

Each value represents the mean of four rats ± standard error.
† Administered orally for 4 weeks.
*P < 0.05, ***P < 0.01, compared with each control value, respectively.
since in the rat liver, four times more L-ascorbic acid is synthesized in the case of D-galactose than when D-glucose is used as a substrate [17].

In rat liver homogenate it was found that continuous PCB administration increases the conversion of D-(1-14C)-galactose to D-(144C)-glucuronic acid. The observed increase in the group of 100 mg/kg/day was statistically significant (Table 4). On the other hand, the formation of L-ascorbic acid from D-glucuronolactone in the liver homogenate was essentially unchanged by PCB administration. In addition, dehydroascorbate activity was not significantly altered with PCB administration. These results suggest that the increase in L-ascorbic acid contents in the liver may be due to activation of the biosynthesis at the steps of galactose to D-glucuronic acid, and not to changes in the catabolism of L-ascorbic acid per se.

**DISCUSSION**

One of the important findings in this study is that continuous administration of PCB induces a significant increase of L-ascorbic acid as well as a drastic decrease of pyridoxal phosphate (PAL-P) in the liver.

The administration of 10 and 100 mg/kg/day of PCB for 4 weeks induced a significant fall in the PAL-P content of the liver, whereas that in the brain was found to be essentially unchanged. Previous studies [1] indicated that the highest levels of PCB are found in adipose tissues and levels in the liver and kidney are also high in these animals. On the other hand, PCB content in the brain was found to be low, although it was higher than that found in the blood. These results suggest that the lack of effect of PCB on the cerebral content of PAL-P may be a simple reflection of the low level of penetration of this compound into the brain. It has been reported, however, that in B₆ deficient rats, no significant changes in cerebral and cardiac B₆ contents are observed despite the occurrence of a significant fall in hepatic B₆ content [21]. This fact also suggests another possibility, that cerebral B₆ is unaltered primarily by PCB.

**TABLE 4. Effect of continuous oral administration of PCB on conversion of D-(1-14C)-galactose to D-(144C)-glucuronic acid by rat liver homogenate**

|          | % Conversion* (Mean±S.E.) |
|----------|--------------------------|
| Control  | 1.21 ± 0.20              |
| PCB (10 mg/kg/day)  | 1.65 ± 0.11              |
| PCB (100 mg/kg/day) | 2.26 ± 0.29*             |

* Each value represents the mean of three rats ± standard error.

* Administered orally for 4 weeks.

* P<0.05, compared with the control value.

% Conversion = \[
\frac{D-(144C)-glucuronic acid count obtained}{total 14C count recovered} \times 100
\]
It is well known that PAL-P is a biologically active form of vitamin B6 and plays an important physiological role as a cofactor for various B6 enzymes. The facts that vitamin B6 deficiency induces various skin diseases, and alterations in hepatic level of vitamin B6 may be an important factor for regulating physiological levels of this substance in the body [22], suggest that the observed decreases in hepatic PAL-P content may be related to the occurrence of well known skin damages due to PCB intoxication [8–10].

In contrast with the significant decrease in hepatic PAL-P contents, L-ascorbic acid contents in the liver, kidney, lung and testis showed a significant increase following continuous administrations of PCB. In the rat liver, it was also found that this increase of L-ascorbic acid is due to the activation of biosynthesis at the steps of galactose to D-glucuronic acid, and not to changes in the catabolism of L-ascorbic acid.

It is well established that L-ascorbic acid plays an essential role in the metabolism of various cells. Involvement of this substance in the biosynthesis of collagen [23], metabolisms of protein [24], carbohydrate [25], and lipid [26], regulation of adrenal cortical functions [27], occurrence of scurvy, and other physiological functions have been reported. In the case of liver, however, possible involvements of L-ascorbic acid and its metabolic intermediate such as UDP-glucuronic acid in the processes of drug metabolism and/or detoxication must also be considered. The present data indicate that continuous administration of PCB induces the activation of biosynthesis at the steps of galactose to D-glucuronic acid, in which the formation of UDP-glucuronic acid is involved. Considering the fact that PCB is excreted in urine as a glucuronic acid conjugate [28], the observed increase in hepatic metabolism of ascorbic acid may also be related to an adaptive increase of detoxifying mechanisms at the stage of PCB intoxication.

The present results indicate that continuous administration of PCB induces significant changes in the metabolism and/or content of pyridoxal phosphate and L-ascorbic acid in various organs. Since these changes were detected following the administration of a rather large dose of PCB, significance of these findings in the pathogenesis of PCB intoxication is uncertain. It is emphasized, however, that PCB is capable of directly affecting the metabolism of these vitamins. Detailed enzymatic mechanisms underlying these changes remain to be elucidated.

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