FURTHER STUDIES ON THE REDUCTION OF VITAMIN A CONTENT IN THE LIVERS OF RATS GIVEN POLYCHLORINATED BIPHENYLS\textsuperscript{1,2}

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Summary Further investigations on the reduction of vitamin A content in the liver of rats fed a 0.1\% PCB diet were conducted. The first experiment, in which rats were fed a 0.1\% PCB diet for 8 weeks and vitamin A in the liver was determined at 2-week intervals, suggested that a significant decrease of vitamin A in the liver might occur within 2 weeks of PCB ingestion. In the second experiment a significant reduction of vitamin A content per gram of liver, but not per whole liver, in rats fed a 0.1\% PCB diet was observed on the 3rd day of PCB ingestion, and then on the 6th day the difference between the control group and the PCB-fed group became much more remarkable. But thereafter no further reduction was seen, indicating a lower limit of vitamin A concentration in the liver of rats fed PCB. It was found that retinol binding protein in the serum of rats fed the 0.1\% PCB diet decreased to one-half that of the control group on the 10th day of PCB ingestion, suggesting also a marked reduction in serum vitamin A level. Another experiment revealed that a decrease in hepatic vitamin A occurred even at low PCB levels, but serum phospholipid did not respond at all to any PCB level examined until 7 days after PCB ingestion began. The mechanisms of sensitive response of vitamin A in the animals fed PCB are briefly discussed.

\textsuperscript{1} Polychlorinated Biphenyls Toxicity And Nutrition VI. Polychlorinated Biphenyls Toxicity And Vitamin A (4).
\textsuperscript{2} The abbreviation PCB is used for polychlorinated biphenyls.
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A close interaction between PCB toxicity and vitamin A has been elucidated in the past few years. Villeneuve et al. (1) reported that vitamin A concentration in the liver was lower in rabbits receiving Aroclor 1254 for 4 weeks than in control ones.

The present authors have previously reported that rats fed for 6 weeks a 0.1% PCB diet supplemented with 3,400 IU of vitamin A showed better growth than those fed a 0.1% PCB diet without the supplementation (2). The effect of vitamin A supplementation was substantiated by the fact that vitamin A concentration in the liver of rats fed a 0.1% PCB diet for 42 days was reduced to approximately one-fifth that of the control group (3). The authors also observed that some characteristic symptoms appeared in rats fed a vitamin A deficient diet with PCB by the 4th week of feeding, while no such sign was noted in rats fed either a vitamin A deficient diet alone or a 0.1% PCB diet alone by the 7th week of feeding (3). The nature of the symptoms was strongly suggestive of a vitamin A deficiency itself. However, these symptoms were suspected to be due to PCB poisoning, according to Tanaka et al. (4).

The previous experiments, therefore, were made to determine whether these symptoms were a characteristic phenomenon of PCB poisoning or were based on a vitamin A deficiency (5). The experimental results clearly demonstrated that a large part of the symptoms was based on a vitamin A deficiency. Thus, the authors concluded that PCB acts to accelerate vitamin A deficiency in the animals or to make them deficient in vitamin A.

This paper deals with further investigations of the interaction between PCB toxicity and vitamin A.

**EXPERIMENTAL**

Weanling male rats of the Sprague-Dawley strain were housed individually in wire cages maintained in a laboratory with 12 hr of light and 12 hr of darkness. The temperature in the laboratory was controlled at 22°C or so. The composition of the basal diet (control) used was the same as in the previous experiment (2). The PCB used in this experiment was purchased from Wako Pure Chemical Inc. It was a mixture of the isomers, of which tetrachloride was dominant. According to each experimental design, the appropriate amount of PCB was added to 100 g of the basal diet in substitute for an equal part of sucrose. All diets used in this experiment were expected to contain 300 IU of vitamin A per 100 g diet, according to the vitamin content of the vitamin oil used (2). The initial vitamin A content per 100 g diet was estimated as 280 IU for the control diet and 297 IU for the PCB diet.

The diets were prepared every 10 to 14 days and kept in a refrigerator except at feeding time. Food and water were given ad libitum throughout the experiments. Food consumption and body weight were recorded three times per week.
At the end of the experiment the food cups were removed at 7 a.m. After fasting for 7–8 hr, the animals were sacrificed by decapitation. Blood was collected for serum lipid analysis. The liver was quickly removed and weighed. The liver and serum were stored in a freezer maintained at −20°C until the determinations were performed. Serum phospholipid was determined by the Fiske and Subbarow method (6) after extraction with a mixture of ethylalcohol and ether (3:1). Hepatic vitamin A was measured by a colorimetric method using a mixture of antimony trichloride and glycerol-1,3-dichlorohydrin after saponification with KOH and separation of the unsaponifiable matters (7). Retinol-binding protein in serum was measured by single radial immunodiffusion according to the method of Mancini et al. (8).

RESULTS AND DISCUSSION

In the first experiment, changes in hepatic vitamin A content of rats fed a 0.1% PCB diet for 8 weeks were investigated. The rats were killed in the 2nd, 4th, 6th, and 8th weeks after PCB ingestion began. The results are shown in Table 1 and Fig. 1.

Table 1. Changes in diet intake, body weight gain, liver weight, and serum phospholipid content in rats fed a 0.1% PCB diet.

| Group          | Feeding period (week) | Diet intake cumulated (g) | Body weight gain (g) | Liver weight (g) | Liver weight per 100 g body weight (g) | Phospholipid in serum (mg/dl) |
|----------------|-----------------------|---------------------------|---------------------|------------------|----------------------------------------|-------------------------------|
| Control        | 2(6)*                 | 179±4**                   | 84±4                | 7.31±0.28        | 4.90±0.12                              | 136±16                        |
|                | 4(6)                  | 448±20                    | 189±7               | 11.77±0.72       | 4.56±0.18                              | 192±36                        |
|                | 6(6)                  | 707±28                    | 256±10              | 14.74±0.89       | 4.38±0.20                              | 183±13                        |
|                | 8(6)                  | 1102±67                   | 333±13              | 14.91±0.83       | 3.71±0.11*                             | 175±8                         |
| 0.1% PCB       | 2(6)                  | 140±6*                    | 38±4*               | 10.33±0.54*      | 9.74±0.24*                             | 211±22*                       |
|                | 4(6)                  | 331±17*                   | 101±11*             | 16.28±1.06*      | 9.67±0.22*                             | 314±18*                       |
|                | 6(6)                  | 612±29*                   | 135±10*             | 18.37±1.28*      | 8.95±0.26*                             | 304±18*                       |
|                | 8(6)                  | 872±24*                   | 170±13*             | 20.40±1.38*      | 8.52±0.13*                             | 382±14*                       |

* Figures in parentheses are the numbers of rats used.
** Mean ± S.E.
* Significantly different from control at each corresponding feeding period, P<0.05.
* Significantly different from 2 weeks’ feeding period in each group, P<0.05.

Although the diet intake in the 0.1% PCB diet group was approximately 80% of that in the control group, body weight gain in the PCB group was about one-half that in the control group. Elevation of serum phospholipid and liver enlargement were also observed in the PCB-fed group, as in the previous study (3). Vitamin A content per gram liver of rats fed the 0.1% PCB diet decreased to al-
most one-fifth that of the control group in the 2nd week after PCB ingestion began, and remained at a low level until the 8th week, while vitamin A content per gram liver of the control group was constant until the 6th week after feeding started and thereafter increased.

On the other hand, vitamin A content per whole liver of the control group continued to increase up to the 8th week, but in the PCB-fed group little change was observed during the entire experimental period except for a slight increase seen in the 4th week. This result suggests that the decrease of vitamin A content in the liver of rats fed a 0.1% PCB diet may occur at a much earlier time, probably within 2 weeks after PCB ingestion begins.

Therefore, the following experiment was attempted to determine when the vitamin A content in the liver began to decrease, and to determine whether the decrease of vitamin A content in the liver of rats fed a 0.1% PCB diet was caused by PCB itself or by less vitamin A intake as a result of a decrease in food intake. According to the measured rates of destruction of vitamin A in the diet during storage in a freezer, as shown in Fig. 2, there was no difference between the vitamin A deterioration curve of the control diet and that of the PCB diet. This indicates that vitamin A may not be specifically destroyed by coexistence with PCB in the diet.

The cumulated vitamin A intake in both groups, calculated from daily food intake and the deterioration curves of vitamin A, is shown in Table 2 and Fig. 3. No difference in the cumulated vitamin A intake between the control group and the PCB group was seen up to the 6th day of PCB ingestion, but thereafter the
Vitamin A content per gram liver of rats fed the 0.1% PCB diet was found to be less than that of the control animals on the 3rd day of PCB ingestion; on the 6th day the difference between the control group and the PCB-fed group became much more conspicuous, but thereafter no more reduction was seen, indicating a lower limit of vitamin A concentration in the liver of rats fed PCB (Fig. 3).

On the other hand, vitamin A content per gram liver in the control group did
not alter much during the 2 weeks. It was found that a converse change in total vitamin A content in the liver of both groups occurred during the 2 weeks. The total vitamin A content in the control group clearly increased, while that in the PCB-fed group had diminished significantly by the 6th day and thereafter remained at the 6th-day level.

The liver weight in both groups approximately doubled during the 2 weeks' feeding, as shown in Table 2. However, the increase of liver weight in the PCB-fed group was much greater than that in the control group. This indicates that the liver development in the control group was normal, but the liver in the PCB-fed group was abnormally enlarged due to PCB. Therefore, the sharp increase of vitamin A content in the whole liver of the control group is found to be based on normal development of the liver in young rats. On the other hand, the vitamin A content in the whole liver of PCB-fed group decreased in spite of abnormal enlargement of the liver. Again, there was not so much difference in vitamin A intake between the two groups, as stated above. Thus, it is concluded that a major cause of decrease in the hepatic vitamin A content is not the smaller vitamin A intake, but rather PCB ingestion itself.

Figure 4 shows that retinol-binding protein in serum of rats fed a 0.1% PCB diet decreased to one-half that of the control group on the 10th day of PCB in-
gestion. It is well established that vitamin A circulates in the blood of humans and rats as a complex with a specific α-globulin, retinol-binding protein (RBP), which is synthesized in the liver (9, 10).

Fig. 4. Retinol binding protein (RBP) and phospholipid in serum of rats fed a 0.1% PCB diet. △ phospholipid, control group; ▲ phospholipid, PCB group; □ RBP, control group; ■ RBP, PCB group. (RBP measurement was done by Drs. Muto and Shidoji.)

SMITH and GOODMAN (11) reported that conditions wherein serum RBP levels are low are marked by a concomitant decrease in serum vitamin A levels. Therefore, a significant decrease of serum RBP levels in rats fed a PCB diet may reflect a marked reduction in serum vitamin A levels. MUTO et al. (12) clearly demonstrated that the lowered serum RBP levels in vitamin A-deficient rats are due to the unavailability of the ligand followed by a decreased secretion of the complex into blood. They also pointed out that the failure of retinol-RBP complex formation is due to the lack of vitamin A in the liver, but not to the inhibition of RBP synthesis, and that the intrahepatic pool of the protein (preformed apo-RBP) is always readily available for the formation of retinol-RBP complex. However, the question of whether the synthesis of RBP may be affected by PCB ingestion remains to be clarified.

There were no significant differences in serum phospholipid concentration between the control and the PCB groups during the 10 days' feeding. However, a rather lower tendency was seen in the serum phospholipid concentration in the PCB-fed group on the 3rd and the 6th days, while it tended to go up thereafter.

A decrease in the amount of hepatic vitamin A and serum retinol-binding protein occurred before an elevation of serum phospholipid concentration, which was one of the biochemical phenomena introduced by PCB ingestion (2, 4). The lowering of amount of vitamin A in the liver was likely to occur at almost the
same time as the enlargement of the liver, which developed immediately after PCB ingestion. Anyway, it seems that vitamin A responds to PCB much more sensitively than serum phospholipid.

This was further confirmed by another experiment, in which hepatic vitamin A content in rats fed on diets containing different levels of PCB was measured on the 7th day of PCB ingestion. The results are shown in Table 3. It was found that the decrease of hepatic vitamin A occurred even at low levels, but the serum phospholipid concentration showed no response to any PCB level examined until day 7.

Table 3. Hepatic vitamin A content and serum phospholipid concentration in rats fed on different levels of PCB for one week.

| Group   | Numbers of rats | Weight gain (g) | Liver weight (g) | Liver weight per 100 g body weight (g) | V.A content IU/g liver | Serum phospholipid (mg/dl) |
|---------|-----------------|-----------------|-----------------|---------------------------------------|------------------------|---------------------------|
| Control | 7               | 51 ± 3.9        | 7.78 ± 0.61     | 4.57 ± 0.22                           | 28.1 ± 1.0             | 177 ± 13                  |
| 0.02% PCB | 7             | 37 ± 3.4*        | 9.68 ± 0.33*    | 6.24 ± 0.09*                          | 13.3 ± 1.1*            | 170 ± 10                  |
| 0.05% PCB | 8             | 36 ± 3.0*<sup>a</sup>,<sup>b</sup> | 11.95 ± 0.46*<sup>a</sup>,<sup>b</sup> | 7.77 ± 0.17*<sup>a</sup>,<sup>b</sup> | 7.3 ± 0.9*<sup>a</sup>,<sup>b</sup> | 180 ± 10                  |
| 0.1% PCB | 7              | 26 ± 1.6*<sup>a</sup>,<sup>c</sup> | 12.86 ± 0.41*<sup>a</sup>,<sup>c</sup> | 8.93 ± 0.15*<sup>a</sup>,<sup>b</sup>,<sup>c</sup> | 7.3 ± 0.5*<sup>a</sup>,<sup>b</sup> | 181 ± 14                  |

* Significantly different from control, P < 0.05.
<sup>a</sup> Significantly different from 0.02% PCB, P < 0.05.
<sup>b</sup> Significantly different from 0.05% PCB, P < 0.05.

It has been demonstrated that amount of vitamin A decreases not only in animals given PCB but also in animals given pesticides such as DDT (13) and dieldrin (14). On the other hand, it is also well known that drug metabolizing enzymes are induced in animals given those compounds. These facts may suggest that under conditions in which drug metabolizing enzymes are induced, the reduction of vitamin A commonly occurs. LITTERST et al. (15) demonstrated that disruption of normal enzyme activity occurred even at dose levels that had no effect on liver weights or on liver: body weight ratios. This may suggest that the reduction of vitamin A proceeds even at dose levels that have no effect on liver weights. Although the mechanisms of reduction of vitamin A in animals given PCB or DDT are not yet clear, it is presumed that microsomal drug metabolizing enzymes may be associated with the reduction of vitamin A.

Liver microsomes are known to catalyze the hydroxylation of various lipid-soluble drugs (16) as well as a number of endogenous compounds such as steroids (17) and fatty acids (18–20).

The liver microsomal enzyme system involved in the hydroxylation of various substances is NADPH- and O<sub>2</sub>-dependent (16), and has a heme protein component, cytochrome P-450 (21).

Das et al. (20) found that treatment of rats with repeated injections of pheno-
barbital caused an increase in the liver microsomal hydroxylation of heptane and laureate parallel to the rates of the oxidative demethylation of testosterone. They also observed that this increase was paralleled by a similar rise in the content of cytochrome P-450 and in the activity of the NADPH-cytochrome C reductase in the liver microsome. Furthermore, the activity of microsomal cytochrome P-450 systems has been shown to increase after in vivo administration of PCB (22, 23) as well as certain other drugs (24). Fujita et al. (22) clearly demonstrated that cytochrome P-450 enzyme activity reached a maximum level on the 6th day of PCB ingestion, and thereafter the activity decreased gradually. On the contrary, the authors found that vitamin A content in the liver of rats fed PCB reached a minimum level on the 6th day of PCB ingestion.

It seems that the recent progress in the investigations of hydroxylation reaction catalyzed by cytochrome P-450 provide an important key to solving the mechanism of vitamin A reduction in animals given PCB or DDT.

Strobel and Coon (25) first proposed that the hydroxylation reaction catalyzed by a microsomal cytochrome P-450 is coupled with a superoxide generating system (xanthine oxidase) in the presence of phosphatidylcholine. This proposal by Strobel and Coon has been supported by research findings over the past five years (26). On the other hand, Fridovich and his co-workers (27, 28) presented a very important fact: that $O_2^-$ and $H_2O_2$, generated by xanthine oxidase, can react to produce $OH$, $HO^-$ and $O_2$ by the Haber and Weiss reaction (29). It is known that the hydroxy radical ($HO^-$) and the singlet molecular oxygen ($O_2$) are the powerful oxygens. Therefore, it is presumed that these active oxygens which occur in the process of the hydroxylation reaction, coupling with a superoxide generating system, may be associated with the reduction of vitamin A in the liver of rats fed PCB, but further investigations of this problem will be conducted in the future.

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