An attempt was made to elucidate the interaction between PCB toxicity and vitamin A. The effects of vitamin A addition or vitamin A deficiency in altering the toxicity of dietary PCB were examined. Rats fed a 0.1% PCB diet supplemented with 3,400 IU of vitamin A for 6 weeks showed better growth than those fed a 0.1% PCB diet only. However, rats given a vitamin A deficient diet with 0.1% PCB showed a significant growth retardation than those given a 0.1% PCB diet only.

All rats which were fed a vitamin A deficient diet with PCB closed their eyes 50% or entirely in the last period of the experiment, but such symptom was not seen in the rats given a 0.1% PCB diet only or a vitamin A deficient diet.

Vitamin A content in the liver decreased significantly with 0.1% PCB administration. It was found that a larger supply of dietary vitamin A than the level contained in the basal diet is required to prevent, to some extent, the growth retardation by PCB administration and to sustain the same level of vitamin A content in the liver of rats fed the same 0.1% PCB diet as that of basal diet group.

It was concluded that animals fed PCB require more vitamin A than usual. This suggests the possibility that vitamin A may play a role in the detoxication of PCB.

Environmental contamination with toxic chemicals is becoming a serious problem throughout the world including Japan which has experienced some unfortunate instances of health hazards involving toxic chemicals.

It is well-known that PCB, which exhibits a marked toxicity for mammals, is one of the sources of environmental contamination (1–3). Several investigators (4–7) have reported that intoxication with PCB at a sufficiently high level of ingestion was marked by growth inhibition or weight loss, an enlargement of
the liver relative to body weight, and an increase of serum lipids level in the test animals. However, very little attention has been paid to the nutritional status and its possible interaction with a toxic stress of PCB. Villeneuve et al. (8) demonstrated that the concentration of liver vitamin A was lower in animals receiving Aroclor 1254 than in control animals. On the other hand, it has been known that an increased intake of vitamin A is beneficial to mitigating the poisonous effects of sodium benzoate (9) and bromobenzene (10).

These findings seemed to indicate that there might be some interaction between PCB toxicity and dietary vitamin A.

Therefore, authors have studied on the effect of vitamin A supplementation on the toxicity of dietary PCB and clarified the fact as reported in the previous paper (11) that the supplementation of vitamin A showed the partial protective effect against the growth retardation by the PCB toxicity when vitamin A was added about ten times as much as the level contained in the basal diet.

In the present study, the further investigation was carried out to elucidate 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 animal laboratory was maintained near 22°C. The composition of the basal diet was (%): milk casein 20.0, sucrose 63.0, soybean oil 9.0, vitamin oil 1.0 (one gram of this oil contains 300 IU of vitamin A-acetate, 30 IU of vitamin D₃ and 10 mg of α-tocopheryl-acetate), vitamin mixture (12) 0.85, choline-HCI 0.15, mineral mixture (12) 4.0 and cellulose flour 2.0. One hundred mg of PCB, which was enough to cause growth retardation in the rats, was added to 100 g of the basal diet in substitute for an equal part of sucrose (11). (PCB used in this experiment was purchased from Wako Pure Chemical Inc.). It was a mixture of the isomers, of which tetrachloride was dominant. An appropriate amount of vitamin A-acetate was added to 100 g of the PCB diet for each experimental design. Vitamin-free casein (Wako Pure Chemical Inc.) was directly used without further purification for the vitamin A-deficient group. The diets were prepared every two weeks and kept in a refrigerator except at feeding time. Six to eight rats of each group were fed the experimental diet. Food and water were given *ad libitum* throughout the experiment. Food consumption and body weight were recorded 3 times per week. At the end of the experiment the food cups were removed at 7:00 a.m. After fasting for 7–8 hr, the rats were sacrificed by decapitation. Blood was collected for serum lipid analysis. Liver was quickly removed, weighed and stored in a freezer maintained at –20°C. Within a few days after sacrifice liver lipids were extracted with a chloroform-methanol mixture (2:1) to prevent hydrolysis of the lipids. Serum and liver extracts were
stored in a freezer until the determination was performed.

Since it has been known that not only do triglyceride (6, 11) and cholesterol (6, 13) in the serum increase on PCB administration, but also serum phospholipid does (6, 11). Phospholipid was selected as one of the biochemical indicators of PCB toxicity in the present experiments.

Phospholipid was determined by the Fiske and Subbarow method (14) after washing extracts by the Folch method (15). Liver vitamin A was determined by a colorimetric method using a mixture of antimony trichloride and glycerol-1, 3-dichlorohydrin following saponification in KOH (16).

RESULTS AND DISCUSSION

The first experiment was carried out to reconfirm the necessity of vitamin A for the partial protective effect against growth retardation by PCB toxicity which was shown in the previous report (11). Rats were fed for 6 weeks on a diet of PCB containing a small amount and a large amount of vitamin A, namely 3 IU and 3,400 IU per 100 g of diet. The body weight gain, liver weight, serum phospholipid concentration, and vitamin A content in the liver were measured. Figure 1 shows the growth curve. A significant body weight gain was found in the rats fed the vitamin A supplemented PCB diet when compared with those given the PCB diet, though the growth of the former group was inferior to that of the control group.

Animals fed a vitamin A-deficient diet with PCB showed a prominent growth retardation and two out of 6 rats of this group died in the last week of the experi-

![Fig. 1. Effect of vitamin A on growth of male rats fed 0.1% PCB diet (mean of 6 rats).](image)
ment. On the other hand, the growth of the vitamin A-deficient group was almost equal to that of the control group, although the growth tended to slow down slightly as compared with the control group in the last week of the experiment. Moreover, no symptom of vitamin A deficiency has been detected in this group. It seems that a good weight gain in the vitamin A-deficient group would be due partly to the trace amount of vitamin A remaining in the vitamin-free casein and partly to the fact that the rats used were comparatively so old that it was too late to make vitamin A deficiency.

As shown in Fig. 2, all rats which were fed a vitamin A-deficient diet with

![Fig. 2. Comparison of PCB toxicity symptom in rats fed 0.1% PCB diet with and without vitamin A for 6 weeks. A, control; B, 0.1% PCB; C, D, V.A deficient diet + 0.1% PCB.](image)

PCB closed their eyes 50% or completely in the last period of 6 weeks feeding. But such eye symptoms were not observed in the group given 0.1% PCB or in the vitamin A-deficient group until the end of experiment. According to the view of TANAKA et al. (6), this symptom is a likely indication of PCB poisoning. But it should be noted that this symptom is very similar to that of vitamin A deficiency. Therefore, further investigation will be required to clarify the reasons of this phenomenon exactly. However, so far as this experiment concerned, it may be inferred that a small amount of vitamin A in the diet accelerates the PCB toxicity or PCB accelerates vitamin A deficiency.
PCB TOXICITY AND VITAMIN A

Table 1. Effect of PCB on food intake and liver weight in rats fed experimental diets with and without vitamin A.a

| Groupb | Food intake (g/day/rat) | Liver weight (g) | Liver weight per 100 g body weight (g) |
|--------|------------------------|-----------------|---------------------------------------|
| Control (6) | 16.6 ± 0.76 | 15.50 ± 0.90 | 4.33 ± 0.12 |
| 0.1% PCB (6) | 9.8 ± 0.88 | 16.42 ± 1.64 | 9.04e ± 0.30 |
| 0.1% PCB +V.A-acetate (5) | 11.4 ± 0.64 | 19.04 ± 2.16 | 8.57 ± 0.98 |
| V.A deficient (6) | 16.3 ± 0.42 | 12.42e ± 0.32 | 3.69e ± 0.09 |
| V.A deficient +0.1% PCB (6) | 8.3e ± 0.68 | 10.45e ± 0.42 | 8.91e ± 0.43 |

a Values are mean ± SE.
b Numbers of rats are indicated in parentheses.
c Significantly different from control: p<0.01.

Table 2. Effect of PCB on phospholipid in serum and liver and vitamin A in liver in rats fed experimental diets with and without vitamin A.a

| Groupb | Phospholipid in serum (mg/dl) | Phospholipid in liver (mg/g) | Vitamin A g liver (IU) | Vitamin A whole liver (IU) |
|--------|-------------------------------|-------------------------------|------------------------|---------------------------|
| Control (6) | 204 ± 15 | 28.2 ± 0.9 | 26.7 ± 4.3 | 422 ± 76 |
| 0.1% PCB (6) | 468e ± 24 | 35.5e ± 0.8 | 5.1e ± 0.8 | 82e ± 14 |
| 0.1% PCB +V.A-acetate (5) | 356e ± 44 | 38.3e ± 0.6 | 136.0e ± 5.1 | 2574e ± 263 |
| V.A deficient (6) | 151d ± 12 | 32.4 ± 0.8 | 2.0e ± 0.6 | 20e ± 6 |
| V.A deficient +0.1% PCB (6) | 399e ± 36 | 42.1e ± 2.4 | 2.4e ± 0.5 | 30e ± 6 |

a Values are mean ± SE.
b Numbers of rats are indicated in parentheses.
c,d Significantly different from control: c, p < 0.01; d, p < 0.05.
e Significantly different from 0.1% PCB: p < 0.01.

Food intake and liver weight were as shown in Table 1. Serum and liver phospholipid concentration and liver vitamin A content were as shown in Table 2. Similar results were obtained in liver weight relative to body weight, and in serum and liver phospholipid concentration in rats fed a PCB diet as in the former experiment.

Vitamin A supplementation could not prevent an enlargement of liver and an increase of serum phospholipid, both of which are regarded as typical symptoms of PCB poisoning.

It was also observed in this experiment that liver vitamin A content of the rats fed a PCB diet decreased significantly as compared with control. A very small amount of vitamin A remained in the liver of the vitamin A-deficient diet group and the vitamin A-deficient diet with PCB group, while in the group given
a diet supplemented with 3,400 IU of vitamin A a considerable amount of vitamin A was stored in the liver. This result was not inconsistent with the result of D. C. VILLENEUVE et al. (8) who demonstrated that the concentration of liver vitamin A in rabbits receiving Aroclor 1254 for 28 days was about half that of control.

The authors observed that vitamin A concentration in the liver of rats fed a 0.1% PCB diet for 42 days was about one-fifth that of the control. The food intake of the PCB diet group, as shown in Table 1, remained at almost 60% of that of the control group. Therefore, it would be reasonable to presume that the heavy decrease in liver vitamin A concentration in the PCB fed group obtained by the authors was due largely to PCB itself, rather than to the smaller vitamin A intake introduced by decreased food intake.

The second experiment was designed to further confirm the partial protective effect of vitamin A against growth retardation caused by PCB administration in the previous experiment.

The effect of vitamin A on the growth of rats fed a PCB diet was examined at various levels of this vitamin. 300 IU (basal), 1,000 IU, 3,000 IU, and 10,000 IU of vitamin A-acetate were added to every 100 g of a 0.1% PCB diet respectively.

As shown in Table 3, the weight gain of rats fed the PCB diet was significantly inhibited compared with that of the control group for 2 months feeding. An addition of 1,000 IU of vitamin A was not effective for offsetting the weight retardation caused by PCB administration. However, it was found that an addition of vitamin A of over 3,000 IU per 100 g of diet was much more effective. But there was found no difference between the rats fed vitamin A 3,000 IU and 10,000 IU group.

Liver weight, serum phospholipid concentration and vitamin A content in the

| Groupb | Body weight gain (g) | Liver weight (g) | Liver weight per 100g body weight (g) | Phospholipid in serum (mg/dl) | Vitamin A in liver (IU/g) |
|--------|---------------------|-----------------|--------------------------------------|-------------------------------|--------------------------|
| Control (8) | 391 ± 14           | 17.11 ± 0.43    | 3.77 ± 0.09                          | 286 ± 16                      | 33.7 ± 4.3               |
| 0.1% PCB (8) | 152± 10           | 18.42 ± 1.11    | 8.42± 0.22                           | 456± 36                       | 3.7± 1.0                 |
| +V.A, 1,000 IU (8) | 162± 16         | 20.79± 1.54     | 9.14± 0.26                           | 365± 23                       | 9.1± 1.9                 |
| 0.1% PCB | 190± 15            | 21.26± 1.69     | 9.50± 1.25                           | 387± 38                       | 148.3± 8.7               |
| +V.A, 3,000 IU (6) | 190± 11         | 21.95± 0.91     | 8.56± 0.39                           | 454± 61                       | 520.0± 47.8              |
| 0.1% PCB | +V.A, 10,000 IU (7) | 190± 11         | 21.95± 0.91                           | 8.56± 0.39                    | 520.0± 47.8              |

a Values are mean ± SE.

b Numbers of rats are indicated in parentheses.
c,d Significantly different from control: c, p < 0.01; d, p < 0.05.
e,f Significantly different from 0.1% PCB: e, p < 0.01; f, p < 0.05.
liver were as shown in Table 3. The symptom of PCB toxicity appeared in liver size and serum phospholipid concentration as well. Liver weight relative to body weight and phospholipid concentration in all of rats fed PCB increased significantly compared with control. Vitamin A content in the liver of rats fed a 0.1% PCB diet decreased significantly in comparison with the control group. Vitamin A content in the liver of rats fed 1,000 IU of vitamin A per 100 g of diet with PCB was almost the same as that of the 0.1% PCB diet group.

It was found that when vitamin A was added at the level of 3,000 IU per 100 g of 0.1% PCB diet, vitamin A content in the liver of this group was higher than in the basal diet group.

Corresponding to the increase of vitamin A in the diet, vitamin content in the liver increased even though PCB was administered. It seems to indicate, as far as the present experimental conditions, that vitamin A supplementation of 1,000 IU per 100 g of 0.1% PCB diet was not so enough to maintain vitamin A content in the liver of PCB-fed animals as vitamin A level in the liver of control group or to prevent the growth retardation by PCB administration. These results may indicate that vitamin A of more than 1,000 IU and less than 3,000 IU per 100 g of diet is required to prevent to some extent the growth retardation and to sustain the same level of liver vitamin A content in the 0.1% PCB diet group as in the control group. However, this does not mean an actual requirement of vitamin A in the rats fed a PCB diet, because of the loss of vitamin A in the diet must be considered. The another experiment (17) which determined the loss of vitamin A in the diet during two weeks storage in a freezer showed a high rate of destruction of vitamin A, calculated as about 75% of the initial content. Therefore, it is understood that the actual requirement for preventing the growth retardation to some extent and maintaining the vitamin A content in the liver of rats fed a PCB diet at the same level as that of the control group will be lower than the level above mentioned.

At any rate, it is apparent that animals fed a PCB diet require more vitamin A than usual. This indicates that vitamin A may play a role in the detoxication of PCB.

It is well known that toxic chemicals such as dibenzanthracene (18), bromobenzene (9, 19), sodium benzoate (8), nitrite (20), aflatoxin (21), DDT (22–24), dieldrin (25) influence vitamin A metabolism in animals. These results strongly suggest that vitamin A is implicated detoxication mechanisms of toxic chemicals.

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