High-Fat Diet Enhances Accumulation of Hexachlorobenzene in Rat Dams and Delays Its Transfer from Rat Dams to Suckling Pups through Milk

Yoko Nakashima,*,† Saeko Ohsawa,‡ and Sachie Ikegami‡

Department of Human Life and Culture, Seitoku University, 531 Sagamidai, Matsudo-city, Chiba 271-8555, Japan, and Division of Food Science, The National Institute of Health and Nutrition, 1-23-1 Toyama, Shinjuku-ku, Tokyo 162-8636, Japan

Effect of diets on the distribution and transfer of hexachlorobenzene (HCB) from dams to fetuses and suckling pups was investigated. In pregnant rats, the amount of HCB accumulated in fat tissues of the high-fat diet group was higher than that of the control diet group ($P < 0.05$). The amounts of HCB in fetuses of the high-fat and control diet groups were estimated to be about 0.28 and 0.12% of the dam's total intake during pregnancy, respectively. In both groups, a large proportion of HCB in dams disappeared during lactation period and was transferred to their pups through the milk. In the pups of the high-fat diet group, the amount of HCB in stomach contents was lower immediately after birth and decreased slowly compared with that in the control diet group during lactation. These results showed that a high-fat diet reduced the speed of the transfer of HCB from the dams to their suckling pups through milk.

Keywords: *Rats; pregnancy; lactation; hexachlorobenzene; high-fat diet*

INTRODUCTION

Many lipophilic organochlorine chemicals, such as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), polychlorinated biphenyl (PCB), 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane (DDT), and hexachlorobenzene (HCB), are transferred from mothers to fetuses through the placenta and to suckling pups through the mother's milk (Ando, 1978; Ando et al., 1985; Kodama and Ota, 1980; Siddiqui et al., 1981). As these chemicals are widely distributed in the global ecosystem, most people are exposed to these chemicals, especially through the consumption of fish, meat, and milk. After absorption, these chemicals tend to accumulate in the fat tissues for a long time without being metabolized (Takayama et al., 1991; Miyata, 1993; Paul, 1993).

In a previous paper, we reported that HCB, a stable, lipophilic organochlorine chemical, was transferred from rat dams to suckling pups through the milk in the early days after birth and that the excretion of HCB into milk was so rapid that the guar gum-induced acceleration of its excretion from the dams was masked (Nakashima et al., 1997, 1998). We have also observed that the metabolism and excretion of the relatively metabolizable pentachlorobenzene (PECB) was increased in young rats by the restricted feeding, a fish oil diet, and a viscous indigestible polysaccharide diet (Ikegami et al., 1994; Umegaki et al., 1993, 1995). This enhanced metabolism and excretion of PECB was due to the small mass of fat tissue that resulted from those treatments. These results suggest that a diet or food components which alter the fat tissue mass might affect the distribution, metabolism, and accumulation of the organochlorine chemicals. Dale et al. (1962); Mitjavila et al (1981), and Wyss et al. (1982) have reported that the amount of highly lipophilic compounds in fat tissue decreased with food restriction or starvation and that their mobilization from fat tissues, metabolism, and excretion were accelerated at the same time.

Because dietary fat influences fat tissue mass, it was considered that a high-fat diet might influence the mobilization, metabolism, and excretion of lipophilic compounds. In this study, therefore, we fed dams a high-fat diet during pregnancy and lactation and examined the influence of such a diet on the transfer of HCB fed to and accumulated by dams during pregnancy to their suckling pups through milk. The high-fat diet was prepared according to the previous work done by Lanoue et al. (1992) and Lanoue and Koski (1994) (low-carbohydrate diet). As their measurements of milk composition indicated significant changes in milk carbohydrate and lipid concentrations, it was expected that the high-fat diet would influence the metabolism and accumulation of HCB in dams as well as its transfer to suckling pups.

In this experiment, we used HCB as an example of a stable lipophilic environmental pollutant. HCB is a stable chlorinated hydrocarbon and has been detected in the placenta, maternal blood, milk, and cord blood of humans (Ando et al., 1985). Pregnant rats were treated with the minimum level of HCB (35.1 nmol/100 g of diet, 10 μg/100 g of diet) that would subsequently produce a detectable organ concentration of HCB. Therefore, it was expected that the biological effect of HCB on the dams and newborns would be very weak. In this paper, we will describe the influence of a high-fat diet on the distribution of HCB in the organs and tissues of pregnant and nursing rats as well as its transfer to the

* Author to whom correspondence should be addressed (telephone 0473-65-1111; fax 0473-63-1401; e-mail yokon@seitoku.ac.jp).

† Seitoku University.

‡ The National Institute of Health and Nutrition.

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Table 1. Compositions of the Experimental Diets

ingredient	control diet[a]	high-fat diet[b]
casein (g/100 g)	20.0	11.0
L-cystine (g/100 g)	0.3	0.3
cornstarch (g/100 g)	39.7486	
dextrin (g/100 g)	13.2	
sucrose (g/100 g)	10.0	
glucose (g/100 g)		15.0
soybean oil (g/100 g)	7.0	30.644
cellulose (g/100 g)	5.0	38.3046
AIN mineral mixture[c] (g/100 g)	3.5	3.5
AIN vitamin mixture[d] (g/100 g)	1.0	1.0
choline bitartrate (g/100 g)	0.25	0.25
tert-butylhydroquinone (g/100 g)	0.0014	0.0014
HCB (nmol/100 g)	35.1	35.1

[a] AIN-93G. [b] Carbohydrates and casein in the high-fat diet were substituted for soybean oil and glucose with minimal but adequate levels. [c,d] Mineral and vitamin mixtures were based on the AIN-93 formulation.

fetuses through the placenta and to suckling pups through the mother's milk.

MATERIALS AND METHODS

Materials. HCB was purchased from Tokyo Kasei Kogyo (Tokyo, Japan) and recrystallized three times by methanol (purity 99%). Other chemicals were purchased from Wako Pure Chemical Industries (Osaka, Japan). Diet components were purchased from Oriental Yeast (Tokyo, Japan).

Diets. The compositions of the control and high-fat diets are shown in Table 1. The control diet composition was based on the AIN-93 G diet (American Institute of Nutrition, 1993). Basically, the glucose and protein contents of the high-fat diet were based on previous work done by Lanoue et al. (1992) and Lanoue and Koski (1994). Carbohydrates and casein in the high-fat diet with minimal but adequat levels of carbohydrate (15%) and protein (11%) were substituted for soybean oil and glucose. Cellulose was added to the high-fat diet to maintain a constant metabolizable energy concentration. Both diets supplied 3.95 kcal/g of diet. The diets containing HCB were prepared by dissolving HCB (3.51 nmol/L of ethanol) in soybean oil.

Animals. Twenty sperm-positive pregnant rats of Sprague-Dawley strain (10 weeks old) were commercially obtained from Japan Clea (Tokyo, Japan) on day 5 of pregnancy. They were housed individually in plastic cages in a room kept at a constant temperature (23 ± 1 °C) and illuminated according to a 12-h light/dark cycle. Rats were given free access to food and distilled water. Dams and suckling pups were weighed, and daily food intake was measured at least four times weekly during the experimental period.

Twenty pregnant rats were divided into five groups of four rats each. One group was fed the diet without HCB during pregnancy and lactation. HCB was given only during pregnancy to two groups in the control diet and to two other groups in the high-fat diet. Among the HCB-fed groups, those in one of the control groups and in one of the high-fat groups were killed on the day before parturition. In the remaining groups, the litters were culled to 10 pups each within 24 h of birth, and the dams were fed either the control or high-fat diet without HCB during lactation. Two pups from each litter on days 2, 5, 11, and 16 after birth and all dams on day 16 after parturition were anesthetized with ether and killed by cardiac puncture. All procedures were in accordance with the guidelines of the National Institute of Health and Nutrition.

Sample Collection and Analysis. All rats were killed by cardiac puncture, and blood was collected with heparinized syringes. Livers, kidneys, and fat tissues of all rats, stomachs of suckling pups, and placentas and fetuses of pregnant rats were removed, weighed, and then stored at −20 °C.

Blood (0.2−3 mL) was mixed with 1−5 mL of distilled water. Organs were homogenized with 4 volumes of water. HCB in the samples was extracted with *n*-hexane. To extract HCB, fat tissues were homogenized with 25 volumes of *n*-hexane. The *n*-hexane extracts were centrifuged at 600*g* for 5 min. The *n*-hexane layer was concentrated if necessary and was cleaned by Florisil column chromatography (0.5 g of Florisil layered on 0.2 g of $NaSO_4$). The column was eluted with 5 mL of *n*-hexane. The eluate was evaporated, and its volume was approximately adjusted with *n*-hexane. HCB was analyzed using a Shimadzu PARVUM QP-5000 gas chromatograph/mass spectrometer (Shimadzu, Kyoto, Japan). The fused silica capillary column DB-624 (0.25 mm × 60 m) (J&W Scientific, Folsom, CA) was used at a column temperature of 250 °C with 20 mL/min helium as carrier gas.

Statistical Analysis. Data are presented as individual group mean ± SEM. Statistical analysis was conducted by one-way ANOVA or, as in the case of time response curves, by two-way ANOVA. Differences in mean values among groups were tested by Duncan's multiple-range test. Student's *t* test was used for all pairwise comparisons. Differences were considered significant at $P < 0.05$. The Yukmus computer program (Yukmus, Tokyo, Japan) was used for statistical analysis of the data.

RESULTS

Body and Organ Weights of Dams. The high-fat diet did not influence the body weight, the weights of the liver and perirenal fat tissue of pregnant rats, and the fetal weight (Table 2). However, the placenta weight of pregnant rats given the high-fat diet was significantly higher than that of rats given the control diet ($P < 0.05$). No significant differences in body and liver weights were observed among the nursing groups. However, the perirenal fat tissue weight of nursing rats fed the high-fat diet was significantly higher than that of the nursing groups fed the control diet with or without HCB ($P < 0.05$). The mean body weight and perirenal fat tissue weight of the two pregnant groups were predictably higher than those of the three nursing groups; however, no significant difference in the liver weight was observed between these pregnant and nursing groups.

Body and Organ Weights of Suckling Pups. On days 2, 5, 11, and 16 after birth, the body weight of suckling pups nursed by dams fed the high-fat diet was significantly lower than that of suckling pups in the groups fed the control diet with or without HCB ($P < 0.05$) (Table 3). No significant difference in body weight was observed between the suckling pups in the control diet groups with or without HCB from day 2 to day 11. However, the body weight of suckling pups in the control diet group with HCB was lower than that of the pups in the group without HCB on day 16 ($P < 0.05$). On days 2 and 11 after birth, the stomach content weights of suckling pups in the high-fat diet group were significantly lower than those of suckling pups in the control diet groups with or without HCB ($P < 0.05$). On days 5, 11, and 16 after birth, the liver weight of suckling pups in the high-fat diet group was significantly lower than in both of the control diet groups ($P < 0.05$). On days 11 and 16 after birth, the liver weight of suckling pups in the control diet group with HCB was lower than that of the pups in the group without HCB ($P < 0.05$). On day 16 after birth, the perirenal fat tissue weight of suckling pups in the high-fat diet group was significantly lower than that in the control diet group without HCB ($P < 0.05$).

Food and HCB Intake and HCB Levels of Pregnant Rats. Food and HCB intakes from day 5 of pregnancy to the day before parturition were, respectively, 288 ± 13 g and 101 ± 5 nmol in the pregnant rats fed the control diet with HCB and, respectively, 276 ± 18 g and 97 ± 6 nmol in those fed the high-fat diet

Table 2. Body and Organ Weights of Pregnant and Nursing Rats Fed the Control or High-Fat Diet Containing HCB at 35.1 nmol/100 g of Diet before Parturition and Weights of their Fetuses[a]

	pregnant dams[b]		nursing dams[c]		
	control diet + HCB	high-fat diet + HCB	control diet − HCB	control diet + HCB	high-fat diet + HCB
body wt (g)	372 ± 21[a]	354 ± 21[a]	302 ± 11[b]	309 ± 16[b]	309 ± 15[b]
liver (g)	14.1 ± 0.4	13.1 ± 0.9	14.1 ± 0.8	14.0 ± 0.3	11.6 ± 0.4
perirenal fat tissue (g)	6.93 ± 1.68[a]	7.21 ± 1.60[a]	1.69 ± 0.41[c]	1.24 ± 0.23[c]	3.10 ± 0.21[b]
placenta (g)	0.59 ± 0.02[a]	0.50 ± 0.02[b]			
fetus wt[d] (g)	3.52 ± 0.26	3.09 ± 0.25			

[a] All values represent mean ± SEM; $n = 4$ in the mother groups. Within a row, values not sharing a superscript letter are significantly different at $P < 0.05$. [b] On day 1 before parturition. [c] On day 16 afer parturition. [d] Number of fetuses of pregnant groups; control (11 + 12 + 16 + 7), high-fat (15 + 10 + 14 + 11).

Table 3. Body and Organ Weights of Suckling Pups Nursed by Dams Fed the Control or High-Fat Diet Containing HCB at 35.1 nmol/100 g of Diet before Parturition[a]

diet	HCB	*n*	body wt (g)	stomach content (g)	liver (g)	perirenal fat tissue (g)
on day 1[b]						
control	−	40	6.30 ± 0.21	ND	ND	ND
control	+	40	6.26 ± 0.11	ND	ND	ND
high-fat	+	40	5.98 ± 0.29	ND	ND	ND
on day 2						
control	−	8	7.44 ± 0.30[a]	0.62 ± 0.06[a]	0.28 ± 0.03	ND
control	+	8	7.63 ± 0.21[a]	0.58 ± 0.03[a]	0.32 ± 0.02	ND
high-fat	+	8	6.38 ± 0.19[b]	0.38 ± 0.08[b]	0.31 ± 0.01	ND
on day 5						
control	−	8	13.07 ± 0.48[a]	0.55 ± 0.06	0.50 ± 0.02[a]	0.017 ± 0.002
control	+	8	13.10 ± 0.25[a]	0.44 ± 0.05	0.50 ± 0.01[a]	0.014 ± 0.002
high-fat	+	8	9.68 ± 0.56[b]	0.44 ± 0.08	0.40 ± 0.04[b]	0.016 ± 0.003
on day 11						
control	−	8	31.44 ± 0.60[a]	1.15 ± 0.13[ab]	1.01 ± 0.02[a]	0.16 ± 0.06
control	+	8	27.92 ± 0.63[a]	1.27 ± 0.14[a]	0.88 ± 0.03[b]	0.13 ± 0.04
high-fat	+	7	20.74 ± 2.65[b]	0.98 ± 0.20[b]	0.64 ± 0.09[c]	0.14 ± 0.01
on day 16						
control	−	8	52.78 ± 1.37[a]	1.18 ± 0.10	2.01 ± 0.15[a]	0.34 ± 0.05[a]
control	+	8	48.70 ± 0.63[b]	1.28 ± 0.14	1.71 ± 0.04[b]	0.25 ± 0.01[ab]
high-fat	+	6	43.56 ± 2.54[c]	1.34 ± 0.14	1.59 ± 0.08[c]	0.20 ± 0.03[b]

[a] All values represent mean ± SEM. Values not sharing a superscript letter are significantly different ($P < 0.05$) in the same day after birth. ND, not determined. [b] On day after birth.

Table 4. HCB Levels in the Blood, Liver, Fat Tissues, Placenta, and Fetus of Pregnant Rats Fed the Control or High-Fat Diet Containing HCB at 31.5 nmol/100 g of Diet[a,b]

		diet (HCB)	
	n	control (+)	high-fat (+)
blood (nmol/L)	4	10.83 ± 1.27	10.92 ± 1.50
liver			
concentration (pmol/g)	4	24.54 ± 2.29	29.67 ± 3.30
total amount (pmol/organ)	4	342.9 ± 24.1	380.4 ± 21.7
perirenal fat tissue			
concentrtation (nmol/g)	4	0.99 ± 0.11[a]	1.42 ± 0.11[b]
total amount (nmol/tissue)	4	6.25 ± 1.04[a]	11.30 ± 1.94[b]
subcutaneous fat tissue			
concentration (nmol/g)	4	0.23 ± 0.04[a]	0.57 ± 0.05[b]
placenta			
concentration (pmol/g)	49	13.68 ± 0.45[a]	17.86 ± 0.61[b]
total amount (pmol/tissue)	49	7.85 ± 0.49	8.59 ± 0.63
fetus			
concentration (pmol/g)	49	2.78 ± 0.17[a]	5.99 ± 1.84[b]
total amount (pmol/ all fetuses)	49	99.82 ± 0.66[a]	197.14 ± 1.19[b]

[a] All values represent mean ± SEM. Within a row, values not sharing a superscript letter are significantly different at $P < 0.05$. [b] On day 1 before parturition.

with HCB. Therefore, there were no significant differences in food and HCB intakes between the pregnant rats fed the control and high-fat diets.

The HCB concentration was the highest in the fat tissues of the pregnant rats on the day before parturition (Table 4). Therefore, large amounts of HCB had accumulated in the fat tissues of the pregnant rats during the pregnancy. The HCB concentrations in the perirenal and the subcutaneous fat tissues and the total amount of HCB in the perirenal fat tissue of the pregnant rats fed the high-fat diet were significantly higher than those of the pregnant rats fed the control diet ($P < 0.05$). However, no significant differences in the HCB concentration in the blood and the concentration and the total amount of HCB in the liver were observed between the pregnant rats fed the control diet and those fed the high-fat diet. Although the HCB concentration in the placenta of pregnant rats fed the high-fat diet was higher than that of pregnant rats fed the control diet ($P < 0.05$), no significant differences in the total amount of HCB in placenta were observed between these groups.

HCB was also detected in the fetuses of both the high-fat and the control diet groups. However, the HCB concentration in fetuses of both groups was significantly lower than those found in the blood and the placentas of their respective dams ($P < 0.05$). The HCB concentration and the total amount of HCB (the amount of HCB transferred from a dam to her litters) in fetuses in the high-fat diet group was significantly higher than that in the control diet group ($P < 0.05$). On the basis of the ingestion of HCB from day 5 of pregnancy to the day before parturition, the amounts of HCB transferred from dams to their fetuses were estimated to be about 0.28 and 0.12% of the amounts consumed by the dams fed the high-fat and the control diets, respectively.

HCB Levels of Nursing Rats. Whether rats were fed the high-fat or the control diet, the HCB levels in blood, liver, and fat tissues of the nursing dams were significantly lower than those of pregnant rats ($P < 0.05$) (Tables 4 and 5). No significant differences in blood

Table 5. HCB Levels in the Blood, Liver, and Fat Tissues of Nursing Rats Fed the Control or High-Fat Diet Containing HCB at 35.1 nmol/100 g of Diet before Parturition[a,b]

	diet (HCB)		
	control (−)	control (+)	high-fat (+)
blood (nmol/L)	0.25 ± 0.04[a]	0.62 ± 0.42[a]	5.45 ± 0.56[b]
liver			
concentration (pmol/g)	0.44 ± 0.11[a]	0.46 ± 0.15[a]	6.38 ± 0.28[b]
total amount (pmol/organ)	6.12 ± 1.03[a]	6.54 ± 1.26[a]	74.18 ± 3.36[b]
perirenal fat tissue			
concentration (nmol/g)	0.04 ± 0.01[a]	0.12 ± 0.03[b]	0.62 ± 0.05[c]
total amount (nmol/tissue)	0.08 ± 0.03[a]	0.15 ± 0.04[b]	1.93 ± 0.21[c]
subcutaneous fat tissue			
concentration (nmol/g)	0.03 ± 0.01[a]	0.10 ± 0.02[b]	0.48 ± 0.09[c]

[a] All values represent mean ± SEM. Within a row, values not sharing a superscript letter are significantly different at $P < 0.05$. [b] On day 16 after parturition.

and liver HCB levels were observed between the nursing rats fed the control diet with or without HCB. However, the HCB concentration in subcutaneous fat tissue and the concentration and the total amount of HCB in the perirenal fat tissue of the nursing rats fed the control diet containing HCB were higher than those of nursing rats fed the control diet without HCB. Furthermore, the HCB levels in the blood, liver, and fat tissues of the nursing rats fed the high-fat diet were significantly higher than those of the nursing rats fed the control diet with or without HCB ($P < 0.05$) (Table 5). Therefore, the HCB concentration in perirenal and subcutaneous fat tissues and the total amount of HCB in the perirenal fat tissue of the nursing rats fed the control diet with HCB were, respectively, decreased to about 1/8.6, 1/2.2, and 1/43 of the levels at the end of pregnancy during the 16 days of the nursing period. On the other hand, the HCB concentration in perirenal and subcutaneous fat tissues and the total amount of HCB in perirenal fat tissue of the nursing rats fed the high-fat diet were, respectively, decreased to about 1/2.1, 1/1.2, and 1/5.9 of the levels at the end of pregnancy during the 16 days of the nursing period (Tables 4 and 5).

HCB Levels of Suckling Pups. On day 2 after birth, the concentration and the total amount of HCB in the stomach contents in the suckling pups in the control diet group were higher than those in the high-fat diet group ($P < 0.05$) (Figure 1). However, these levels of HCB in the control diet group were decreased rapidly during 16 days of lactation. In contrast, although the HCB concentration in the stomach contents in the high-fat diet group was gradually decreased, no significant difference in the total amount of HCB in stomach contents was observed during the 16 days of lactation. Therefore, on day 16 after birth, both the HCB concentration and the total amount of HCB in the stomach contents were higher in the suckling pups in the high-fat diet group than those of the suckling pups in the control diet group ($P < 0.05$). The concentration of HCB in the blood was only 1/4 to 1/5 of that in the stomach contents (Figure 1). On day 2 after birth, the HCB concentration in the blood of the suckling pups in the control diet group was highest and decreased rapidly from day 2 to day 16. The change of HCB concentrations of the pups during the lactatuin period was small in the high-fat diet group. Therefore, on days 11 and 16 after birth, the HCB concentration in the blood of pups in the high-fat diet group was higher than that in the control diet group ($P < 0.05$).

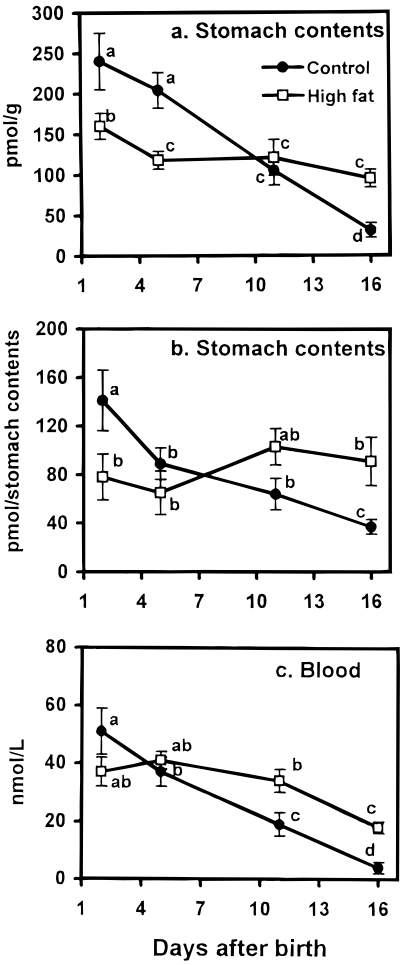

Figure 1. HCB levels in the stomach contents and blood of suckling pups nursed by dams fed the control or high-fat diet containing HCB at 35.1 nmol/100 g of diet during pregnancy and fed the same diet without HCB after parturition: (a) HCB concentration in stomach contents; (b) total amount of HCB in stomach contents; (c) HCB concentration in blood. On days 2, 5, 11, and 16 after birth, two suckling pups from each litter of four dams were killed. Values are expressed as means ± SEM. Values not sharing a letter are significantly different at $P < 0.05$.

The HCB concentrations in the perirenal fat tissue of the suckling pups in the control and the high-fat diet groups were able to be determined on day 5 after birth (Figure 2). The concentrations in the perirenal fat tissue in the both groups were high on day 5. That in the control diet group was more rapidly decreased than that in the high-fat diet group. Therefore, on day 16 after birth, the concentration in the high-fat diet group was significantly higher than that in the control diet group ($P < 0.05$). On the contrary, the total amounts of HCB in perirenal fat tissue in both groups were lower on day 5 than on days 11 and 16 after birth. However, the total amount in the perirenal fat tissue was more rapidly increased in the high-fat diet group than in the control diet group. Therefore, on days 11 and 16 after birth, it was significantly higher in the high-fat diet group than that in the control diet group ($P < 0.05$). On days 11 and 16 after birth, the HCB concentration in subcutaneous fat tissue was higher in the high-fat group than that in the control diet group as well as in the perirenal fat tissue.

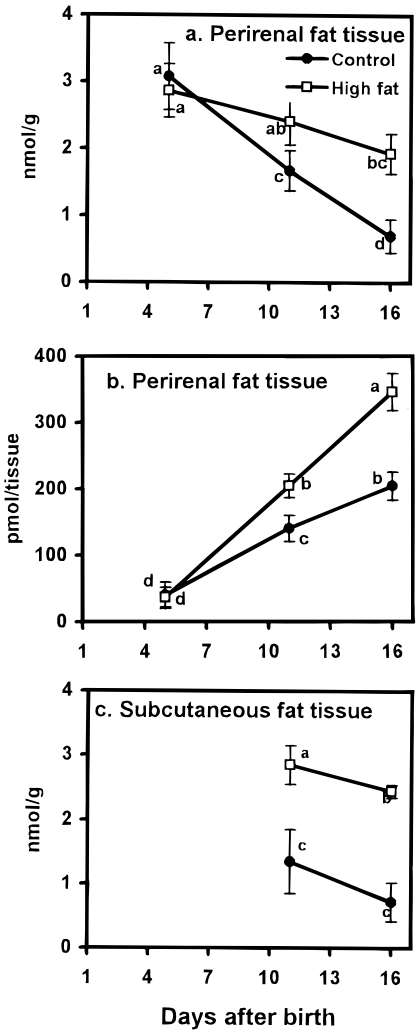

Figure 2. HCB levels in the fat tissues of suckling pups nursed by dams fed the control or high-fat diet containing HCB at 35.1 nmol/100 g of diet during pregnancy and fed the same diet without HCB after partuiriton: (a) HCB concentration in perirenal fat tissue; (b) total amount of HCB in perirenal fat tissue; (c) HCB concentration in subcutaneous fat tissue. On days 2, 5, 11, and 16 after birth, two suckling pups from each litter of four dams were killed. Values are expressed as means ± SEM. Values not sharing a letter are significantly different at $P < 0.05$.

DISCUSSION

In a previous paper (Nakashima et al., 1997, 1998), we have already reported that a large proportion of HCB accumulated in dams disappeared during the lactation period and transferred to their suckling pups through the milk. In this paper, we observed that the amount of HCB accumulated in pregnant rats fed the high-fat diet was significantly higher than that in the control diet. However, immediately after birth, the amount of HCB in the stomach contents of suckling pups nursed by dams fed the high-fat diet was lower than that of suckling pups nursed by dams fed the control diet (Figure 1). Koppe (1995) considered that mobilization of fatty acids from fat tissue would release the therein stored dioxin, which would then be excreted in the breast milk. A low-fat/high-carbonydrate diet (~20% from fat for total energy intake) and a high-fat/low-carbohydrate diet (~50% from fat) were tested for their effects to reduce the concentration of dioxin in human milk. Because no significant influence on the dioxin concentration in breast milk could be found, Koppe concluded that short-term dietary measures would not reduce the dioxin concentration in human milk. However, in his paper, he also showed that although the major part of the fatty acids in the breast milk would be derived from mobilized adipose and hepatic lipids, the contribution of fatty acids derived from dietary lipids could be enhanced, usually by 30%, by using a high-fat diet.

In the present study, no significant differences in body and perirenal fat tissue weights were observed between the pregnant rats fed the high-fat diet and those fed the control diet on the day before parturition (Table 2). However, on day 16 after parturition, although no significant difference in body weight was observed between these two groups of nursing rats, the perirenal fat tissue weight of nursing rats fed the high-fat diet was significantly higher than that of rats fed the control diet. In other words, the decrease of perirenal fat tissue mass after parturition of dams fed the high-fat diet was smaller than that of dams fed the control diet. Furthermore, HCB levels in fat tissues of nursing rats fed the high-fat diet were higher than those of nursing rats fed the control diet (Table 5). On the other hand, the HCB concentrations in the stomach contents and blood of suckling pups from the high-fat diet group were lower than those of suckling pups from the control diet on day 2 after birth. In addition, the decrease of the HCB concentration of suckling pups from the high-fat diet group was slower than that of suckling pups from the control diet group after birth. Therefore, we concluded that the high-fat diet suppressed the mobilization of fatty acids from fat tissue and concomitantly reduced the mobilization of HCB from fat tissues. Furthermore, because the amount of HCB accumulated during pregnancy in dams fed the high-fat diet was higher than that of dams fed the control diet, on day 16 after birth, a large amount of HCB was transferred to their suckling pups from dams fed the high-fat diet.

Ando et al. (1985) reported that HCB was detected in all preparations of human placenta, maternal blood, and cord blood from a group of general Japanese subjects that fetuses were exposed to the lipophilic chlorinated compounds passing through the placental barrier, and that a significant linear correlation existed between the HCB concentrations in the placenta and cord blood. In this study, the pregnant rats fed the high-fat diet accumulated larger amounts of HCB in fat tissues and showed higher HCB concentrations in the placenta compared with the pregnant rats fed the control diet (Table 4). It was considered that the amount of HCB transferred from the pregnant rat fed the high-fat diet to their fetuses was significantly higher than that from pregnant rats fed the control diet. Therefore, the amount of HCB accumulated in fetuses of pregnant rats fed the high-fat diet was higher than that in fetuses of pregnant rats fed the control diet (Table 4).

HCB has been reported to be hepatotoxic and immunotoxic and to affect thyroid hormone homeostasis (Besten et al., 1993; Carlson and Tardiff, 1976; Rush et al., 1983). Besten et al. (1993) reported that female rats receiving a diet containing 0.015% HCB did not show hepatic porphyrin accumulation and urinary porphyrin excretion during the initial 4 weeks, but did show a very slight increase after 10 weeks. In the present study there was no evidence that the small amount of HCB harmed the dams and suckling pups except that, on day

16 after birth, the suckling pups nursed by dams fed the HCB diet had lower body weights than those nursed by dams fed the HCB-free diet. However, the high-fat diet decreased the body weight of suckling pups. The high-fat diet used in this experiment is characterized by low protein and carbohydrate contents. The requirement for dietary protein for pregnant dam has been reported to be lower than that of the growing rats (Karimzadegan et al., 1979). The protein level (11%) of the high-fat diet of Lanoue and Koski (1994) used in this study was slightly lower than the requirement for the weanling rats (13.3% casein) shown by Karimzadegan et al. (1979). However, Lanoue and Koski (1994) observed a significant reduction of milk fat concentration when dams were fed a low-carbohydrate diet throughout pregnancy and lactation, but there were no differences in milk protein, lactose, and glucose concentrations. Therefore, it was considered that the protein level of the high-fat diet was minimal but adequate for the pregnant and nursing dams. The lower fat content observed in milk of dams fed low-carbohydrate diet resulted in lowering the metabolized energy transferred to suckling pups; this might injure the postnatal pup growth.

Although the biological effect of HCB on the dams and suckling pups was very weak in the present study, it is important to address the question of whether prenatal and postnatal exposures to HCB produce long-term harmful effects. Further study will be required to determine the risk factors associated with the transfer and accumulation of these pollutants in breast-fed infants.

In conclusion, this study demonstrates that the amount of HCB accumulated in dams fed the high-fat diet during pregnancy was higher than that of dams fed the control diet. Although prenatal transfer of HCB to rat fetuses was very small, a greater portion of the HCB that accumulated in dams during pregnancy was postnatally transferred to suckling pups through milk immediately after birth. However, the amount of HCB in stomach contents in the pups of the high-fat diet group was lower immediately after birth and decreased slowly compared with that in the control diet group during lactation. These results showed that a high-fat diet reduced the speed at which HCB is transferred from dams to suckling pups through milk.

ABBREVIATIONS USED

DDT, 1,1,1-trichloro-2,6-bis(*p*-chlorophenylethane); HCB, hexachlorobenzene; PCB, polychlorinated biphenyl; PECB, pentachlorobenzene; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin.

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