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Adipose Tissue Content as a Modifier of the Tissue Distribution, Biological Effects, and Excretion of a Hexachlorobiphenyl in C57BL/6J and DBA/JBOMf Mice

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SUMMARY

C57BL/6J (C57) and DBA/JBOMf (DBA) mice were used to study the role of adipose tissue as a modifier of tissue distribution, biological effects, and elimination of a lipophilic foreign chemical, 2,4,5,2′,4′,5′-hexachlorobiphenyl (HCB). As an indication of biological potency of the model compound, the activities of hepatic drug-metabolizing enzymes were determined. DBA mice contained twice as much body fat as C57 mice. Since the highly lipophilic HCB was primarily sequestered by the adipose tissue, DBA mice required greater doses of HCB than did C57 mice to reach similar tissue levels of the chemical. Accordingly, greater HCB doses were required by DBA mice for elevation of drug-metabolizing enzyme activities. Phenobarbital elevated enzyme activities in a similar way in both mouse strains. When the dietary intake of DBA mice was restricted, the body fat content decreased from 15% to 5% of body weight during 1 week. In these animals the tissue accumulation of HCB and enzyme induction resembled the situation in C57 mice fed ad libitum. Highest elevations were seen in the activities of 7-ethoxycoumarin-*O*-deethylase and arylhydrocarbon hydroxylase (EC 1.14.14.2). In addition, the activity of epoxide hydrolase (EC 3.3.2.3) was increased, whereas glutathione *S*-transferase as well as UDP-glucuronosyltransferase (EC 2.4.1.17) activities remained unchanged. The abundant adipose tissue content played no role in the nonresponsiveness of DBA mice to 3-methylcholanthrene since, in contrast to C57 mice, no changes in enzyme activities were detected in DBA mice deprived of food, even after large doses of 3-methylcholanthrene. The adipose tissue content also affected the rate of elimination of HCB. DBA mice excreted smaller quantities of HCB than did C57 mice after equal doses. When, however, fasted DBA mice received HCB, they excreted it at rates similar to those of C57 mice fed ad libitum. In C57 mice, concomitant to the elevation of monooxygenase activities, there was an increase in the rate of excretion of HCB. No such elevation could be seen after a dose that was too small to elevate enzyme activities.

INTRODUCTION

Laboratory mouse strains are commonly divided into two main categories according to their response to induction of microsomal monooxygenase activity by certain foreign compounds. Some mouse strains respond with induction of various drug-metabolizing enzyme activities to exposure of polycyclic aromatic hydrocarbons, whereas other strains fail to respond (1). The prototype of the former is the C57BL/6J strain; of the latter, the DBA/2J strain. Polycyclic aromatic hydrocarbon-responsive and -nonresponsive strains also differ in their susceptibility to many biological effects of halogenated aryl hydrocarbons (2–5).

Recently, Decad *et al.* (6) demonstrated strain differences between C57BL/6J and DBA/2J mice in the tissue distribution and excretion of 2,3,7,8-tetrachlorodibenzofuran. These differences were explained as resulting from the more abundant adipose tissue content of nonresponsive mice. Interestingly, these investigators suggested that dissimilarities in the responses of the two types of mice to halogenated aryl hydrocarbons might be due in part to differences in adipose tissue content.

The aim of the present study was to investigate the role of adipose tissue in modifying the response of C57[1] and DBA mice to lipophilic foreign compounds. In accordance with the results of Decad *et al.* (6), the present study shows that the adipose tissue content of mice

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[1] The abbreviations used are: C57, C57BL/6J mice; DBA, DBA/JBOMf mice; PCB, polychlorinated biphenyl; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; HCB, 2,4,5,2′,4′,5′-hexachlorobiphenyl.

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modulates the tissue distribution and biological effects of HCB. As an indication of biological effects, the activities of various drug-metabolizing enzymes were determined. Moreover, the present study shows how elimination of HCB depends on (a) the adipose tissue content and (b) the elevation of drug-metabolizing enzyme activities in mice.

EXPERIMENTAL PROCEDURES

Materials. HCB was purchased from Ultra-Scientific (Hope, R.I.). 2,4,5,2′,4′,5′-Hexachloro-(U-^{14}C)biphenyl (specific activity 20.7 Ci/mole; radiochemical purity more than 99%) and [^{3}H]styrene oxide (specific activity 15.5 Ci/mole) were from NEN Chemicals (Dreieich, Federal Republic of Germany), [^{3}H]benzo[*a*]pyrene (specific activity 27 Ci/mmole) from Amersham (Buckinghamshire, United Kingdom), and ^{14}C-labeled internal standard from LKB-Wallac (Turku, Finland). 7-Ethoxycoumarin was a generous gift from Dr. A. Zitting, Institute of Occupational Health (Helsinki, Finland). All other chemicals were of analytical grade. The purity of the HCBs was checked by gas chromatography-mass spectrometry by Dr. J. Tarhanen, Department of Chemistry, University of Jyväskylä (Jyväskylä, Finland). No chlorinated dibenzo-*p*-dioxins or dibenzofurans were detected (the detection limit was 0.05 ppm).

Animals and their treatments. Adult male C57 (24.2 ± 3.7 g) and DBA (24.2 ± 3.5 g) mice were used. Mice were allowed access to a standard laboratory diet and tap water ad libitum. Some of the DBA mice were fed a restricted amount of food in order to reduce the amount of adipose tissue. These animals received 1–2 g of pelleted standard food daily until they had lost 24–35% of their body weight, after which time they received 4–5 g of food daily starting 12 hr before treatment of the animals.

HCB was dissolved in corn oil. When [^{14}C]HCB was used, it was dissolved in hexane and added to a corn oil solution of cold HCB. Hexane was evaporated at 37° under nitrogen flow. The HCB solution was injected i.p. (5 ml/kg) into the mice at the indicated doses. The mice were killed 5 days after a single HCB dose. 3-Methylcholanthrene in corn oil was injected i.p. into mice at a dose of either 200 mg/kg (twice) or 80 mg/kg (once), and the animals were killed 1 or 5 days after the last dose, respectively. Control animals received an equal volume (5 ml/kg) of corn oil i.p. Phenobarbital was given in drinking water (0.1% w/v) for 7 days before the mice were killed.

The total daily excreta were collected by housing each animal in a glass metabolic cage. The excreta were dried at 37° and ground to powder with a mortar and pestle.

Preparation of cytosolic and microsomal fractions. The mice were decapitated, and the liver was excised and chilled in ice-cold 0.25 M sucrose solution. A 20% (w/v) liver homogenate was prepared in a 0.25 M sucrose solution (0°) with a Potter-Elvehjem glass-Teflon homogenizer driven by an electric drill at 500 rpm. Microsomes were prepared from postmitochondrial (12,000 × g_{max} for 10 min at 4°) supernatant fluid by the calcium aggregation method (7, 8). The postmicrosomal supernatant (27,000 × g_{max} for 15 min) fluid was saved for determination of glutathione *S*-transferase activity. Protein content was measured by the biuret method (9) with bovine serum albumin as the reference protein.

Enzyme assays. Care was taken to perform all enzyme activity measurements under optimal conditions with respect to incubation time and protein concentration so that the initial velocities were determined.

Microsomal aryl hydrocarbon hydroxylase was assayed radiochemically with 3,4-benzo[*a*]pyrene as the substrate (10).

7-Ethoxycoumarin *O*-deethylation was determined by the method of Ullrich and Weber (11) as modified by Aitio (12).

The activities of microsomal epoxide hydrolase (13) and cytosolic glutathione *S*-transferase (14) were measured with styrene oxide as the substrate.

UDP-Glucuronosyltransferase activity was determined with 4-methylumbelliferone (15) or 2-aminophenol (16) as the aglycone. In order to activate the latent UDP-glucuronosyltransferase, microsomal preparations were treated with digitonin (17). The optimal digitonin concentration and treatment time were carefully determined for the two strains and were found to be 0.67% (0.5–0.8 mg/mg of protein) and 20 min at 0°, respectively, for both strains.

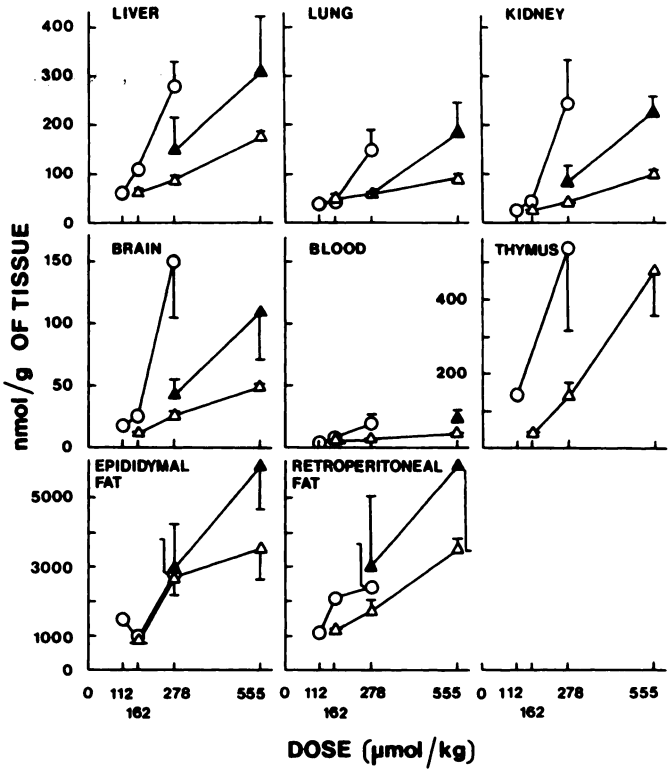

FIG. 1. *Tissue distribution of HCB 5 days after a single i.p. injection in C57 and DBA mice*

HCB was dissolved in corn oil (111.2, 55.6, 32.4, or 22.4 μmoles/ml) and [^{14}C]HCB was added (4.2 μCi/ml). C57 mice (o) received 112, 162, or 278 μmoles/kg (the number of animals was two, two, or five, respectively), and DBA mice (Δ) received 162, 278, or 555 μmoles/kg (three, three, or four animals, respectively) of the compound. Some of the DBA mice (▲) had restricted access to food in order to reduce the body fat content before their treatment with the HCB (278 or 555 μmoles/kg; four or three animals, respectively). The HCB in the tissues was quantitated radiochemically after combustion of the samples as described under Experimental Procedures. The parent compound and the metabolites were not separated. The means ± standard errors of the means are indicated.

TABLE 1

Body fat content of C57 and DBA mice

The body fat of homogenized mice was extracted with diethyl ether and quantitated gravimetrically after solvent evaporation as described under Experimental Procedures. A group of DBA mice had restricted access to food until they had lost 31.0 ± 1.9% of their body weight (see Fig. 3). Other mice were fed ad libitum. The number of animals was three. Values are means ± standard deviation.

Mouse strain	Fat content	Body weight
	% body wt	*g*
C57	7.9 ± 1.5	25.1 ± 1.4
DBA, normal diet	14.7 ± 1.9[a]	25.2 ± 2.3
DBA, fasted	5.1 ± 1.3[b]	16.0 ± 1.0

[a, b] Significantly different from C57 mice (*a*) or from DBA mice on a normal diet (*b*) at $2p < 0.01$ using Student's *t*-test.

TABLE 2

Amount of HCB in adipose tissue of C57 and DBA mice

Subcutaneous, epididymal, and retroperitoneal fat was totally dissected and the HCB content was determined radiochemically after combustion of samples. Values are means ± standard error of the mean.

Dose of HCB	Mouse strain		
	C57	DBA, normal diet	DBA, fasted
μmoles/kg	*% HCB dose in adipose tissue*		
278	16.2 ± 4.4 (9)[a]	28.0 ± 3.3 (7)	13.1 ± 2.5 (4)
555	—	15.6 ± 2.1 (4)	7.4 ± 2.5 (3)

[a] Number of animals in parentheses.

Quantitation of HCB-derived radioactivity in tissues and excreta. Samples of different tissues were excised and weighed immediately after decapitation. The samples were dried at 37° for 24 hr and combusted in a Packard Model 306 sample oxidizer. Five samples of each daily excretus collection were combusted. The formed CO_2, including $^{14}CO_2$, was trapped to Lumasorb II, and Lipoluma was used as the liquid scintillator. Isotope recovery was checked frequently, and was 89–93%. The radioactivity was determined by liquid scintillation counting, and the results were corrected for quenching as well as for recovery with internal standards.

Quantitation of HCB in liver. Hepatic concentrations of HCB were determined by modifying the method described by Karppanen *et al.* (18). One part of the tissue and four parts (w/w) of anhydrous sodium sulfate were homogenized with a glass rod in a large glass tube. The resulting powder was layered into a small glass column and eluted slowly with diethyl ether (15 ml/g of tissue) at room temperature. The eluent was allowed to evaporate at room temperature, and the residue was dissolved in 1 ml of hexane. One milliliter of concentrated H_2SO_4 was added and the mixture was shaken for 10 min, centrifuged at 2000 × *g* for 10 min (4°), and frozen at −70°. The hexane phase was decanted and stored at −70° until analyzed by gas-liquid chromatography. The recovery was determined by analyzing liver samples fortified with known amounts of HCB. Results were not corrected for the recovery (93%).

The gas-liquid chromatographic analyses were performed with a Hewlett-Packard Model 5700 A gas chromatograph equipped with a ^{63}Ni electron capture detector. The carrier gas was nitrogen (99.998% pure). The column used was GCM-079 (Analabs, North Haven, Conn.), 4 feet × ⅛ inch (stainless steel), 2.5% *N,N'*-bis(*p*-methoxybenzylidene)-*α,α'*-bi-*p*-toluidine on Chromosorb W HP, 100/120 mesh. The injector, column, and detector temperatures were 250°, 203°, and 300°, respectively.

Quantitation of total body fat content. Each mouse was cut into small pieces with scissors and homogenized in a small volume (1 ml/5 g) of hexane at 20,000 rpm_{max} (Virtis 45 homogenizer). To one part of the resulting slurry three parts (w/w) of anhydrous sodium sulfate were added and the combination was mixed. The resulting powder was extracted in a Soxhlett apparatus with diethyl ether for 2 hr. Diethyl ether was evaporated *in vacuo* at 30°, and the fat yield was quantitated gravimetrically.

Radioactivity measurements and statistics. Radioactivity was counted in an LKB-Wallac Rack Beta II scintillation counter. Efficiencies for ^3H and ^{14}C counting were 30% and 53–80%, respectively.

Student's *t*-test was used in the statistical evaluation of the results.

TABLE 3

Hepatic drug-metabolizing enzyme activities of C57 and DBA mice exposed to HCB, 3-methylcholanthrene, or phenobarbital

HCB and 3-methylcholanthrene were administered i.p. at the indicated doses. DBA mice were killed 5 days or 1 day after the last dose of HCB or 3-methylcholanthrene, respectively. C57 mice were killed 5 days after the single 3-methylcholanthrene dose. Phenobarbital was given in drinking water as a 0.1% solution for 7 days before the animals were killed. Enzyme activities were determined in freshly prepared microsomes with the exception of glutathione-*S*-transferase, which was assayed in the postmicrosomal supernatant (see Experimental Procedures). Values are means ± standard error of the mean.

Mouse strain and treatment	Arylhydrocarbon hydroxylase	7-Ethoxy-coumarin *O*-deethylase	Epoxide hydrolase	Glutathione *S*-transferase	UDP-Glucuronosyltransferase	
					4-Methyl umbel-liferone	2-Aminophenol
	nmoles/min/g (wet wt)					
DBA, normal diet						
Control	11.2 ± 1.1 (4)[a]	53.0 ± 3.5	47.3 ± 2.3	9,780 ± 220	226 ± 14	
HCB, 555 μmoles/kg	12.4 ± 0.6 (4)	111.5 ± 10.5**	63.6 ± 2.1**	10,550 ± 170*	206 ± 5	ND[c]
DBA, fasted						
Control	10.7 ± 0.9 (3)	66.7 ± 14.6	38.3 ± 7.5	11,080 ± 760	263 ± 34	
HCB, 555 μmoles/kg	19.0 ± 0.3*** (3)[b]	252 ± 29**	65.9 ± 6.3*	12,950 ± 580	306 ± 30	ND
C57						
Control	6.80 ± 0.9 (4)	34.3 ± 4.0	32.3 ± 2.1	14,970 ± 990	263 ± 34	5.21 ± 0.26
3-Methylcholanthrene, 80 mg/kg (one injection)	19.1 ± 3.6* (4)	128 ± 15***	35.8 ± 4.2	12,470 ± 990	379 ± 36	9.47 ± 0.29***
DBA, fasted						
Control	16.1 ± 0.9 (5)	56.5 ± 6.3	26.2 ± 4.2		135 ± 41	
3-Methylcholanthrene, 200 mg/kg (two injections)	17.7 ± 1.0 (7)	85.1 ± 7.0*	27.3 ± 3.1	ND	185 ± 20	ND
C57						
Control	12.4 ± 1.0 (4)	36.4 ± 3.1	23.7 ± 0.9	10,470 ± 430	186 ± 20	4.90 ± 0.63
Phenobarbital	17.0 ± 1.5* (4)	299 ± 61**	66.2 ± 5.3***	23,020 ± 1520***	359 ± 29**	8.72 ± 0.26**
DBA, normal diet						
Control	8.04 ± 0.64 (4)	29.7 ± 1.9	35.1 ± 0.8	10,110 ± 350	209 ± 20	6.62 ± 0.41
Phenobarbital	19.0 ± 2.1** (4)	298 ± 36***	99.8 ± 5.9***	22,000 ± 620***	418 ± 29**	10.1 ± 0.4***

[a] Number of animals in parentheses.

[b] Significance is expressed as follows (Student's *t*-test): *** $2p < 0.001$, ** $2p < 0.01$, * $2p < 0.05$.

[c] ND, Not determined.

RESULTS

In both mouse strains, the adipose tissue sequestered by far the highest concentration of HCB. The adipose tissues of both C57 and DBA mice contained about the same level of HCB, whereas in most other tissues the HCB concentration was strikingly higher in C57 mice (Fig. 1). Adult DBA mice, fed ad libitum, contained approximately twice as much diethyl ether-extractable fat as did C57 mice of the same age and weight, housed under similar conditions (Table 1). The more abundant body fat content of DBA mice sequestered a greater proportion of the HCB dose than did the body fat of C57 mice (Table 2), and thus caused the different tissue distribution.

As positive controls of the effects of foreign compounds on drug-metabolizing enzyme activities, the classical enzyme inducers phenobarbital and 3-methylcholanthrene were administered to mice of both strains (Table 3). 3-Methylcholanthrene treatment of C57 mice elevated hepatic monooxygenase activities as well as the glucuronidation of 2-aminophenol, whereas the activities of epoxide-metabolizing enzymes and 4-methylumbelliferone conjugation remained at control levels. As expected, 3-methylcholanthrene had no effects on the nonresponsive DBA mice. On the contrary, the responses of C57 and DBA mice to phenobarbital were very similar. Activities of all of the studied enzymes (monooxygenases, epoxide metabolizing enzymes, and UDP-glucuronosyltransferase) were markedly elevated in both mouse strains.

Indicating the presence of HCB, the activities of hepatic monooxygenases were elevated in parallel to the tissue content of HCB (Fig. 2). When measured at three time points (2, 5, and 10 days) after a single i.p. injection of 278 μmoles/kg of HCB, the activities of arylhydrocarbon hydroxylase and 7-ethoxycoumarin *O*-deethylase in the livers of C57 mice were highest 5 days after the injection. Similarly, hepatic HCB concentrations were highest at the same time point. With this dose the hepatic HCB concentration of DBA mice was only half that of the C57 mice, and the hepatic monooxygenase activities remained at control levels. After a dose of 555 μmoles/kg, the hepatic HCB concentration of DBA mice was high enough (Fig. 1) to increase 7-ethoxycoumarin *O*-deethylase and epoxide hydrolase activities (Table 3).

When the food intake of DBA mice was restricted in such a way that the animals lost about 30% of body weight during 1 week (Fig. 3), the amount of diethyl ether-extractable fat decreased from 15% to 5% of body weight (Table 1). As an obvious consequence of the decrease in the body fat content, more HCB could be found in most tissues of fasted DBA mice than in tissues of normally fed DBA mice after equivalent HCB doses (Fig. 1). When hepatic HCB levels of DBA mice were increased, elevations of aryl hydrocarbon hydroxylase and 7-ethoxycoumarin *O*-deethylase activities became evident (Table 3). In addition to the monooxygenases, the activity of epoxide hydrolase was enhanced. These changes were somewhat smaller than those caused by phenobarbital in normally fed DBA mice (Table 3). Restricted access to food did not alter significantly the basal activities of hepatic drug-metabolizing enzymes.

In all, the excretion of [^{14}C]HCB-derived radioactivity was slow. Five days after injection of a single dose of 278 μmoles/kg, only 9% of the total dose had been excreted by C57 mice (Table 4). The initial excretion rate by DBA mice was even slower. The excreted radioactivity was not analyzed chemically; therefore, it is not known whether HCB was excreted as such or as its metabolites. In C57 mice treated with 278 μmoles/kg of HCB, concomitant to the elevation of enzyme activities (Fig. 2) there was an elevation of the rate of excretion of [^{14}C]HCB-derived radioactivity (Fig. 4). No such elevation could be seen after a dose that was too small to elevate monooxygenase activities (112 μmoles/kg).

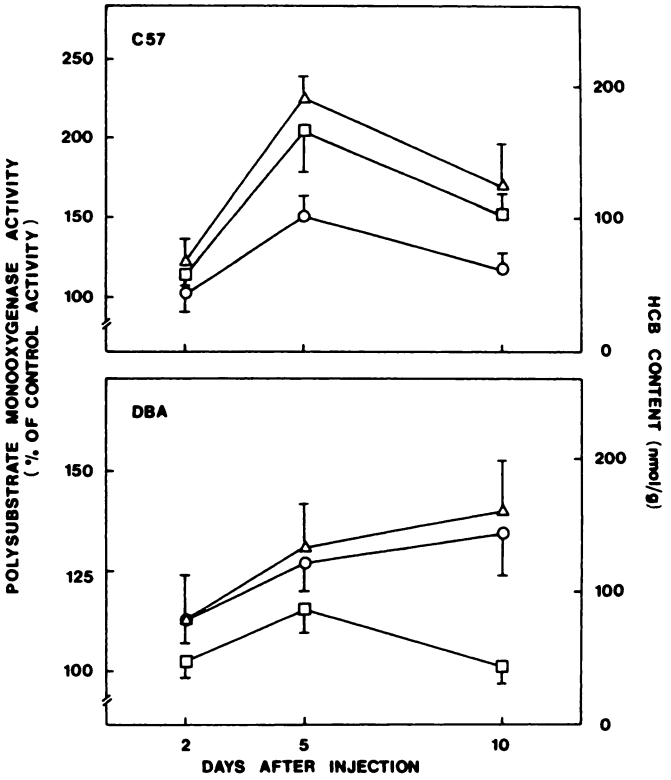

FIG. 2. *Hepatic HCB concentration and polysubstrate monooxygenase activities in C57 and DBA mice at various time points after a single i.p. injection (278 μmoles/kg) of the compound*

Control activity or arylhydrocarbon hydroxylase (○) was 4.63 ± 0.47 nmoles/min/g (wet weight) for C57 and 5.03 ± 0.30 nmoles/min/g (wet weight) for DBA mice. Control 7-ethoxycoumarin *O*-deethylase activity (△) was 30.0 ± 3.4 nmoles/min/g (wet weight) for C57 and 6.71 ± 0.53 nmoles/min/g (wet weight) for DBA mice. The hepatic HCB content (□) was quantitated after diethyl ether extraction as described under Experimental Procedures. Each point represents the mean ± standard error of the mean of four animals.

The amount of adipose tissue also affected the excretion of HCB from the body. In DBA mice with decreased adipose tissue content, the excretion of [^{14}C]HCB-derived radioactivity was faster and resembled the situation in normally fed C57 mice (Fig. 4; Table 4).

DISCUSSION

It is remarkable that, until the study by Decad and coworkers (6), no attention had been paid to the marked difference between the content of adipose tissue in C57BL/6 and DBA/2 mice. Surely this difference will become evident in such investigations only when careful

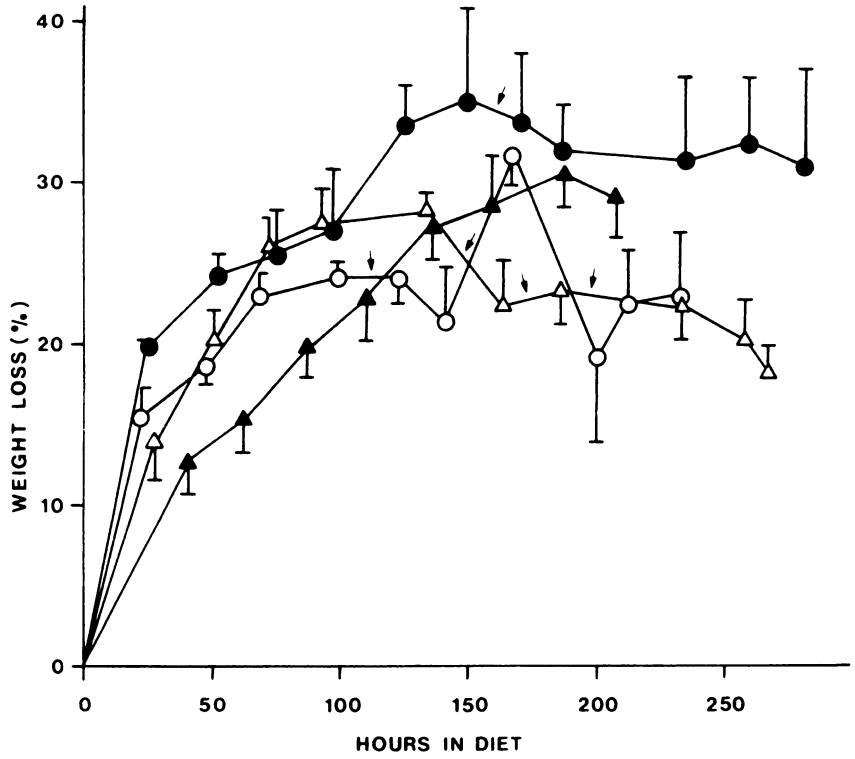

FIG. 3. *Loss of body weight of DBA mice on a diet with restricted food intake*

The mice were given 1–2 g of pelleted food daily. When the animals had lost 24–35% of their body weight they were given 4–5 g of food daily. Two groups of mice received a single i.p. dose of HCB, either 278 (○) or 555 (●) μmoles/kg. Two groups of the mice received 3-methylcholanthrene i.p., either three injections of 20 mg/kg, and were killed 48 hr after the last dose (△), or two injections of 200 mg/kg, and were killed 24 hr after the last dose (▲). The injection times are shown by *arrows*. Each point represents the mean ± standard deviation for 6–12 animals.

postmortem examinations of mice is performed. Restricting the dietary intake of nonresponsive DBA mice resulted in loss of adipose tissue, whereafter the amount of body fat was similar in the two strains of mice. Therefore, the above mouse strains offered an experimental system able to elucidate the role of adipose tissue as a modifier of the tissue distribution, biological potency, and elimination of a lipophilic foreign compound.

It was fundamental to this study to have an exact measure of the total adipose tissue content of experimental animals. Our method, gravimetric determination of body fat after diethyl ether extraction of homogenized mice, proved reproducible. Despite the totally different techniques, our data on the body fat content of C57 and DBA mice are consistent with the data given by Decad *et al.* (6).

HCB was used as a representative of lipophilic xenobiotics since PCBs are a classical example of environmental chemicals which, entering the body, are stored in the adipose tissue (19, 20). The biological effects of PCBs

TABLE 4

Total radioactivity excreted by C57 and DBA mice during 5 days after a single dose of [^{14}C]HCB

The data are from the same animals as in Fig. 4. The indicated doses were administered to mice as i.p. injections. Excreta were collected once daily for 5 days. The radioactivity was analyzed after combustion of the samples, as described under Experimental Procedures. Values are means ± standard error of the mean.

Mouse strain	Dose of HCB	No. of animals	Excreted radioactivity during 5 days	
			Amount	% Dose
	μmoles/kg		*nmoles*	
C57	112	2	208	8.4
C57	162	2	341	9.6
DBA, normal diet	162	3	171 ± 16	4.8 ± 0.5
C57	278	5	598 ± 91	9.2 ± 1.7
DBA, normal diet	278	3	160 ± 17[a]	2.8 ± 0.6[b]
DBA, normal diet	555	4	534 ± 43	4.6 ± 0.5
DBA, fasted	555	3	611 ± 72	7.9 ± 1.2[c]

[a] Significantly different ($2p < 0.01$) from C57 mice at the same dose.
[b] Significantly different ($2p < 0.05$) from C57 mice at the same dose.
[c] Significantly different ($2p < 0.05$) from DBA mice on a normal diet at the same dose.

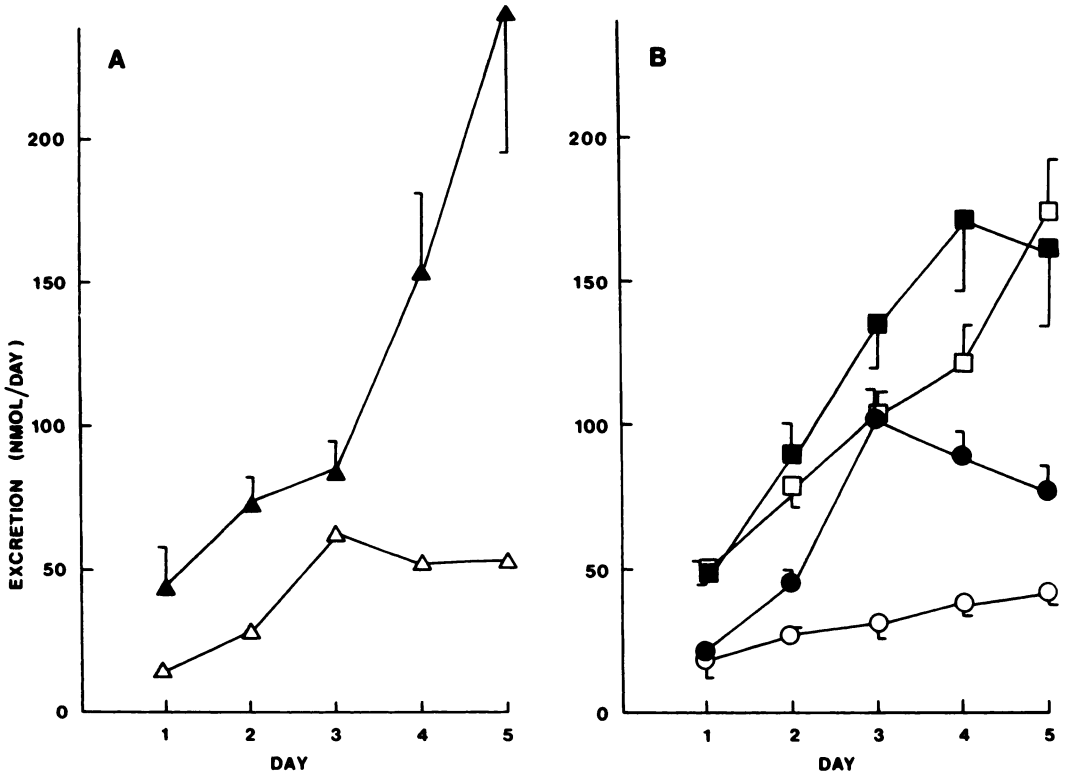

FIG. 4. *Excretion of [^{14}C]HCB-derived radioactivity in C57 and DBA mice after a single i.p. injection of the compound*

C57 mice (A) received either 112 (△) or 278 (▲) μmoles/kg and DBA mice (B) either 278 (○, ●) or 555 (□, ■) μmoles/kg of the compound. Some of the DBA mice (●, ■) had restricted access to food in order to reduce the body fat content of the animals before their treatment with HCB. The data are from the same animals as in Fig. 1. The means ± standard errors of the means are indicated.

have been the object of an ever-increasing number of studies; one of the most studied examples is induction of a great variety of enzymes, especially the drug-metabolizing enzymes (see ref. 21). In the present study, enhancement of various drug-metabolizing enzyme activities was chosen to indicate the potency of the model compound, since other biological effects of HCB do not appear as quickly and are not quantitated as accurately.

HCB is a known representative of PCBs resembling phenobarbital as an inducer of drug-metabolizing enzymes (22). Phenobarbital elevates these enzyme activities in a similar way in both C57 and DBA mice (23, 24), as was confirmed also in this study. Therefore, it is not likely that the different dose requirements for enzyme induction by HCB were due to the nonresponsiveness of DBA mice.

In the present study it was shown that the abundant adipose tissue content of DBA mice sequestered HCB with the consequence that HCB concentrations in most tissues of DBA mice were markedly lower than those in tissues of C57 mice. This is the most likely explanation for the greater HCB doses required for enzyme induction in DBA mice, since elevations of hepatic drug-metabolizing enzyme activities are determined directly by the concentration of HCB in the liver (25). In this way, affecting the tissue distribution of HCB, the amount of body fat modifies the biological potency of this chemical. This view is further supported by the fact that fasted DBA mice, having the same content of body fat as normal C57 mice, behaved more like normal C57 mice with regard to the tissue distribution of HCB and elevation of drug-metabolizing enzymes by HCB.

In general, those of the halogenated aryl hydrocarbons which are the most effective inducers of aryl hydrocarbon hydroxylase also have proved to be the most toxic congeners (5, 22). HCB slightly induces aryl hydrocarbon hydroxylase activity (22), as was seen also in the present study. Nevertheless, as a "phenobarbital-type" congener, HCB belongs to the less toxic class of PCBs (5, 22).

The elimination of HCB was also influenced by the amount of body fat. This was seen as a slower initial excretion rate of radioactive material by DBA mice. Earlier it was shown that fasting of rats previously exposed to PCBs leads to an elevation of the excretion of PCB-derived material (26).

In addition to HCB and 2,3,7,8-tetrachlorodibenzofuran (6), the hepatic TCDD concentration is smaller in DBA mice than in C57 mice after equal dosing (4, 27). In light of the evidence given in the present paper and in the study by Decad *et al.* (6), it is likely that the different doses of halogenated aryl hydrocarbons required to bring about the same response in C57 and DBA mice are to some extent due to different "buffering capacities" of the adipose tissue in the two strains of mice. The final conclusions await studies in which normally fed and fasted DBA mice are compared with regard to their dose responses to 2,3,7,8-tetrachlorodibenzofuran and TCDD. In case of 2,3,7,8-tetrachlorodibenzofuran and TCDD, the effect of adipose tissue may prove less dramatic since it is known that, instead of adipose tissue, liver is the

primary site of accumulation of these chemicals in the body (6, 28). However, it has been shown that TCDD is eliminated two times more slowly by DBA mice than by C57 mice (29). It is important to note that the amount of body fat seems to play no role in the nonresponsiveness of mice to a polycyclic aromatic hydrocarbon, 3-methylcholanthrene. Fasted DBA mice did not respond to 3-methylcholanthrene, even if administered at very large doses.

The "buffering" effect of adipose tissue against the toxic effects of PCBs was demonstrated earlier by Carlson (30). Restricting the food intake of rats previously exposed to PCBs resulted in a more pronounced elevation of drug-metabolizing enzyme activities, probably due to liberation of PCB stores by the mobilization of adipose tissue.

Thus far, the modifying role of adipose tissue has largely been forgotten when the biological effects of halogenated aryl hydrocarbons have been estimated. It is now tempting to speculate whether differences reported in the responses of experimental animals of various species, strains, sexes, and ages to highly lipophilic compounds could be explained in part by differences in the amount of body fat. In addition, when PCB levels in human fat biopsy samples are measured, the total body fat content should be taken into account to avoid erroneous estimation of total body burden.

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