Lipogenic Enzymes of Rat Liver and Adipose Tissue. Dietary Variations and Effect of Polychlorinated Biphenyls

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The lipogenic enzymes fatty acid synthase (FAS; EC 2.3.1.85), citrate cleavage enzyme (CCE; EC 4.1.3.8), malic enzyme (ME; EC 1.1.1.40), glucose-6-phosphate dehydrogenase (G6PDH; EC 1.1.1.49) and 6-phosphogluconate dehydrogenase (PGDH; EC 1.1.1.44) were investigated in liver and in brown adipose tissue (BAT) of Wistar rats under various dietary conditions and in the presence of 15 to 250 ppm (approximately 0.045–0.75 μmol/kg chow) polychlorinated biphenyls (PCBs).

In response to refeeding starved animals, enzyme activities in both tissues increased to above normal levels and thereafter exhibited pronounced oscillations of their activities. The extent of increase depended on the carbohydrate and fat content of the diet. The lipogenic enzymes could be grouped in two categories according to their sensitivity to dietary carbohydrate: FAS and CCE responded faster to smaller changes in dietary composition, while ME, G6PDH and PGDH required larger changes and more time to respond.

Diet-induced alterations of enzyme activities were of the same order of magnitude in liver and BAT. They were age-dependent, being more pronounced in young animals. Independent of the type of dietary manipulations, activities changed in a coordinate fashion, *i.e.,* the changes of the activities of all 5 enzymes occurred at similar ratios to each other with an identical time course.

Feeding PCB-containing diets resulted in a considerable increase of the activities of the lipogenic enzymes in liver, which was significantly greater with ME, G6PDH and PGDH. The effect was dose-dependent but transient. In liver the response to PCB feeding was identical in male and female animals, whereas in BAT lipogenic activities increased in females, but decreased in males.

Refeeding starved animals with a PCB-containing diet led to an additional stimulation of the normal refeeding-induced increase of the enzyme activities in liver and BAT. This PCB-induced increase was 2-fold for FAS and CCE, but up to 15-fold for the other enzymes. All PCB-induced effects were significantly less pronounced in old than in young animals.

In primary hepatocytes activities increased in hormone-free medium in the presence of PCBs. While activity was induced in insuline- and triiodothyronine-containing medium, this increase was significantly greater with PCBs present.

Introduction

PCBs are toxic pollutants of the polychlorinated aromatic hydrocarbon group which, as a result of their presence in numerous industrial processes and products, are ubiquitous in the environment (Safe, 1984). As manufactured for commercial use PCBs are a mixture of isomers containing between 21 and 60% chlorine by weight. There are 209 theoretically possible congeners. PCBs are lipophilic compounds which can bind to hydrophobic domains of proteins or associate with the non-polar moiety of lipids. Their tissue distribution is essentially controlled by the lipid content of a given tissue (Lutz *et al.,* 1977).

Liver and adipose tissue are the main sites for metabolism and storage of PCBs, respectively. In blood PCBs are associated with the lipoprotein fraction, chylomicrons or low density lipoproteins being the carriers for the PCBs (Matthews *et al.,* 1987). Liver is apparently the primary target organ; typical symptoms of PCB exposure are

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hepatomegaly, increased liver lipid content, porphyria, and decreased carcass lipids (Berdanier *et al.*, 1975). Proliferation of smooth endoplasmatic reticulum associated with increased microsomal enzyme activity has been reported (Grant *et al.*, 1974), and this effect is thought to be mediated by a cytosolic receptor, the so-called Ah receptor (Safe, 1984). Brown adipose tissue has been suggested to be a target organ for the toxicity of another typical polychlorinated aromatic hydrocarbon, *viz.*, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) (Rozman, 1984).

Several metabolic systems and a number of enzymes are affected by exposure to PCBs: enzymes of gluconeogenesis, oxidative phosphorylation, microsomal drug metabolism, phospholipid metabolism, triglyceride metabolism, sterol and particularly cholesterol metabolism (for a review see Gamble, 1986).

Lipid synthesis constitutes a major portion of the metabolism in both liver and BAT and the pathway is under delicate dietary and hormonal control. The rate of fatty acid synthesis and the activities of the lipogenic enzymes decrease under conditions of starvation or insulin deficiency or after consumption of a fat-rich diet. Refeeding, administration of insulin or a low-fat, high carbohydrate diet on the other hand will markedly enhance the activities of the lipogenic enzymes and restore the ability of these tissues to form triglycerides from ingested carbohydrate (Wakil *et al.*, 1983).

The present study was performed to elucidate the mutual influences of dietary manipulations and exposure to PCBs on the activities of enzymes of lipogenesis in liver and BAT. While many studies with PCBs were conducted using relatively high dietary concentrations (*viz.* 500 ppm or more), the effects of PCBs described here were observed with concentrations as low as 30–60 ppm PCBs. Doses of more than 250 ppm were not used, as under these conditions feed intake and body weight gain were reduced and the animals were in poor condition.

Experimental

Animals and diets

Wistar rats from our own husbandry were used and maintained on a balanced stock diet (standard diet tpf 1324, Altromin, Lage, Germany). This diet contained 60% cereal-derived carbohydrate mixture, 19% protein, 7% fat and 5% crude fiber, plus vitamins and minerals. Experimental diets used were obtained from ICN Nutritional Biochemicals, Cleveland, Ohio, U.S.A. Their compositions are summarized in Table I.

Clophen A-50 (Bayer AG, Leverkusen, Germany) was used as source of PCBs. It has a chlorine content of 54% and an average molecular weight of 327. A concentration of 100 ppm PCBs in the diet corresponds to approximately 0.30 μmol/kg chow. The main components are pentachlorobiphenyls (45%), tetrachlorobiphenyls (28%) and hexachlorobiphenyls (16%) (Oesterle and Deml, 1984). PCB-containing diets were prepared by adding PCBs, dissolved in ethanol, under stirring to a powdered stock diet (50 ml PCB solution per 500 g of diet). The mixture was then pasted with water, pellets were formed and dried at 25 °C for 3 days. A control diet was prepared identically with ethanol only. In all experiments, the term PCB-free balanced stock diet refers to the latter diet. With 15–250 ppm PCB rats maintained their weight relative to the control animals.

Animals, female or male as indicated, 120–150 g (35–45 day-old), were used throughout. Age-related experiments were performed with animals of 120 g (35 days) and 450 g (250 days). The animals were housed in a temperature- and humidity-controlled room at 25 ± 2 °C on a 12 h light/dark cycle (lights on 06:00–18:00). Except under fasting conditions the animals had free access to feed. For the refeeding experiments the animals were fasted for 3 days and subsequently refed with a diet as indicated.

Tissue sampling and preparation of crude extracts

Livers and interscapular BAT were removed under ether anesthesia between 8 and 9 a.m. and

Table I. Composition of diets.

Ingredients [%][a]		Diet No. 1	2	3	4
Carbohydrate	sucrose	69	68	–	22
	starch	–	–	60	–
Protein		21	18	27	64
Fat		–	8	10	8

[a] Plus vitamins and mineral mix.

rapidly chilled in ice-cold buffer (0.1 M sucrose, 0.05 M KCl, 0.04 M KH_2PO_4, 0.03 M EDTA, pH 7.2). Contaminating tissue was carefully removed from BAT. The tissues were homogenized in the above medium (1 g tissue/3 ml medium) at 4 °C with a Potter Elvehjem homogenizer (20 strokes). Liver homogenates were centrifuged at 20,000×*g* for 30 min, the resulting supernatants were centrifuged at 105,000×*g* for 60 min and the resulting supernatants used as enzyme source. BAT homogenates were centrifuged at 105,000×*g* for 45 min, the clear infranatant was decanted from the fat layer and used as enzyme source.

The lipogenic enzymes were assayed with established spectrophotometrical procedures: FAS (Hsu *et al.*, 1963), CCE (Inoue *et al.*, 1966), ME (Hsu and Lardy, 1967), G6PDH (Löhr and Waller, 1974), and PGDH (King, 1974). Lactate dehydrogenase (LDH) (EC 1.1.1.27) was assayed as described (Bergmeyer and Bernt, 1974). Specific activity is expressed as μmol/min×mg protein at 25 °C. Protein was determined according to Lowry *et al.* (1951), using bovine serum albumin as standard.

All biochemicals, if not stated otherwise, were purchased from Sigma Chemical Company, St. Louis, U.S.A. (Deisenhofen, Germany).

Serum lipids

Blood was obtained from ether-anesthetized animals by cardiac puncture. Serum lipids were analyzed using diagnostic kits as follows: triglycerides (Sigma #320 A), total cholesterol (Boehringer #1442341), and phospholipids (Boehringer #691844).

Hepatocytes

Hepatocytes were prepared and cultured according to Spencer and Pitot (1982). Cells were plated on Petri dishes (10 cm diameter) in 10 ml/dish Ham's F 12 basal medium, containing fetal calf serum (10% v/v) and 1 μM dexamethasone. Cell density at the time of plating was 5×10^6 cells/ml. Induction medium was basal medium plus 1 μM insulin and 10 μM triiodothyronine. PCBs (Clophen A-50), dissolved in DMSO (150 mg/ml) were added at 2 μl/ml medium under sonication (Sonifier B-12, Branson Ultrasonic Corp., Danbury, CT, U.S.A.) prior to the addition of the hepatocytes. To harvest the hepatocytes the incubation medium was removed and the cells were rinsed twice with 0.145 M NaCl. Then 0.5 ml extraction buffer (see above) were added and the cells scraped from the Petri dishes. Each dish was then rinsed with an additional 0.1 ml of the same buffer. The hepatocyte suspension was sonicated (30 s in an ice bath) and subsequently centrifuged at 20,000×*g* for 30 min. The resulting supernatant was used as enzyme source.

Results and Comments

Lipogenic enzymes of liver and adipose tissue

In Table II the activities of the investigated lipogenic enzymes in liver and BAT of young and old female and male rats are summarized. In liver the activities were similar in young animals of both sexes. With increasing age all five activities in liver decreased. The decline was substantial between 35 and 70 days of age, but not as striking at a later age (not shown). The age-related decline

Table II. Activities of lipogenic enzymes in rat liver and brown adipose tissue.

Enzymes	Specific activity [μmol/min × mg protein][a]							
	Liver				Brown adipose tissue			
	Male		Female		Male		Female	
	a Young	b Old	c Young	d Old	e Young	f Old	g Young	h Old
Fatty acid synthase	12	3.2	13	6.1	8.2	3.1	3.8	3.7
Citrate cleavage enzyme	25	9.0	24	14	4.1	0.8	2.2	2.7
Malic enzyme	45	10	39	34	30	14	11	12
Glucose-6-phosphate dehydrogenase	3.8	1.8	3.8	4.2	4.1	2.2	3.2	3.6
Phosphogluconate dehydrogenase	8.5	4.2	13	11	2.2	0.8	1.7	1.4

[a] Each value represents the mean of 4 young (35 days) or 3 old animals (males, 220 days; females, 270 days). Standard errors were 5–15% of mean values and are omitted for clarity.

was more pronounced in females than in males (Table II). G6PDH and PGDH, which supply NADPH for fatty acid synthesis through the pentose phosphate pathway, decreased only slightly in the liver of females (Table II).

The age-related decline of lipogenic activities might be related to the obesity which generally develops in older rats. The activity of CCE decreased with age when animals were fed *ad libitum*, but remained unchanged when a weight-maintaining amount of feed was given (Hoffmann *et al.*, 1980). This implies that the decline of CCE activity is related to the development of obesity rather than to the process of aging *per se*. After refeeding starved animals the amount of mRNA for lipogenic enzymes in young rats was approximately 7-fold higher than in old animals and roughly paralleled the lipogenic activity (Katsurada *et al.*, 1982). Thus, the age-dependent decrease of the lipogenic activities might be caused by a decrease of mRNA transcription.

The activities of the lipogenic enzymes in BAT were generally lower than those in liver (Table II) with the exception of G6PDH activity, which was similar in both tissues. In female BAT the activities were even lower than in males but an age-related decline was observed only in males.

Dietary variations of the activities of lipogenic enzymes in liver and brown adipose tissue

Coordinate adaptive changes

It is known that the lipogenic enzymes in liver and BAT will decrease upon starvation and increase again upon refeeding (Gibson *et al.*, 1972; Wearie and Kanagabasai, 1982). Fig. 1 summarizes the changes of the enzyme activities in BAT; patterns of change were essentially identical in liver. The activities of lipogenic enzymes decreased in both tissues during the 3 day starvation period, and the decline was more distinct in liver (65–80% below fed controls) than in BAT (45–65%). In both tissues activities of FAS and CCE decreased more than those of the other enzymes (data not shown). After 3 days starvation the animals were refed with a high-sucrose, fat-free diet. The individual activities of the lipogenic enzymes in BAT (Fig. 1) and liver were induced to various extent, displaying a steep initial increase of activity to levels much above those observed in non-starved control animals. Overshooting of the activities of lipogenic enzymes upon starvation/refeeding has been described before (Muto and Gibson, 1970; Gibson *et al.*, 1972, Yagil *et al.*, 1974). The extent of overshoot depended on the preceding period of starvation, *i.e.* it was less pronounced with only 1 or 2 days of starvation. Initial peaking of enzyme activities could vary between 48 and 72 h.

The initial overshoot was followed by pronounced oscillations of the activities (Figs. 1 and 3). Similar overshooting and subsequent oscillations of enzyme activities as a response to starvation/refeeding has been described for G6PDH (Yagil *et al.*, 1974). The reason for this oscillation is as yet unknown, but may include a signal which affects a cytoplasmic repressor controlling the rate of mRNA synthesis and/or degradation or may include a direct effect of carbohydrate on the synthesis/degradation of the lipogenic enzymes.

The type of dietary manipulation exerted a strong influence on the peak value of the initial overshoot of enzyme activities as well as duration and amplitude of the subsequent oscillations in response to starvation/refeeding. Table III compares the enzyme activities at the first peak of induction (72 h) after the onset of refeeding animals with various diets (*cf.* Table I). Activity of CCE is depicted as representative for all five enzymes. The strongest increase of CCE activity was observed with a high-sucrose, fat-free diet (diet 1 of Table 1). Dietary fat inhibited this increase of ac-

Table III. Effect of starvation and subsequent refeeding diets of different composition on the activity of brown adipose tissue citrate cleavage enzyme. Male animals, fed the standard rodent chow, were starved for 3 d and subsequently refed for 3 d (first maximum, see Fig. 1) with the specified diets No. 1–5. Numbers indicate diets listed in Table I. Diet No. 5 is the balanced stock diet of standard feeding (see Experimental section). Values are means of 4 animals ± SEM.

Treatment of animals	Citrate cleavage enzyme (specific activity)				
Standard chow fed	4.3 ± 0.5				
Starved (3 days)	1.6 ± 0.2				
	Diet No.				
	1	2	3	4	5
Refed 3 days with diets No. 1–5	49.2 ± 4.6	38.3 ± 3.9	21.8 ± 1.9	14.2 ± 1.3	9.4 ± 1.1

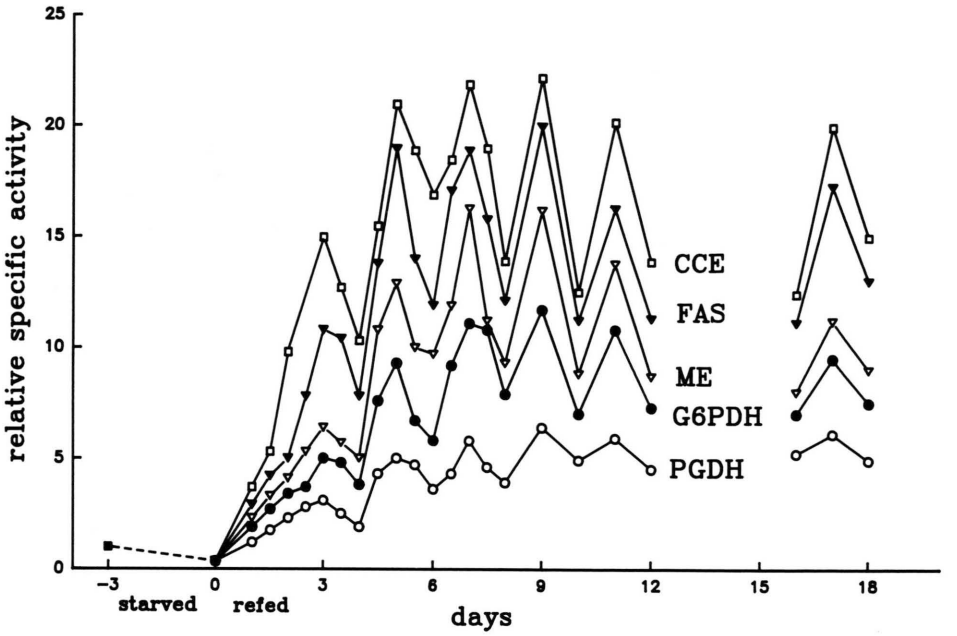

Fig. 1. The effect of starvation and subsequent refeeding on the activities of the lipogenic enzymes in brown adipose tissue. Female animals, previously fed the balanced stock diet were starved for 3 d and then refed with a high-sucrose, fat-free diet (diet 1 of Table I). Specific activity of the enzymes in animals fed the balanced stock diet (basal activity) was set at 1. For absolute values of basal enzyme activities see Table II g. The symbol at time zero represents the specific activity of the different enzymes after the 3 d starvation period which was decreased 65, 68, 58, 50 and 45% for CCE, FAS, ME, G6PDH and PGDH, respectively. Values are means of 4 animals. Standard errors (6–15%) omitted for clarity.

tivity (compare diets 1 and 2). Both the amount of carbohydrate in the diet (compare diets 2 and 4) as well as its source (sucrose *vs.* starch, compare diets 2 and 3) influenced the pattern of response, sucrose being more efficient than starch. Refeeding with a diet high in sucrose or without fat resulted in strong oscillations, which persisted for more than 16 days (Fig. 1). Refeeding diets which contained a less easily digestible carbohydrate or fat also resulted in a similar overshoot of enzyme activity, but oscillations were less pronounced and of shorter duration (see also Fig. 3).

When, after a period of standard feeding a certain diet, the diet was replaced by another one of different composition, this caused a fast adaptation of the enzyme activities to levels characteristic for the new diet (data not shown). Pronounced oscillations were seen when the new diet contained less fat or more valuable carbohydrate (sucrose instead of starch), leading to an increase of the lipogenic enzymes to higher levels of activity.

The adaptive responses of these enzymes in liver and BAT displayed different sensitivity thresholds towards induction by dietary sucrose. The minimum and maximum amounts of sucrose that can stimulate a refeeding-induced increase and elicit a peak effect, respectively, after 3 days of starvation, were determined. In both cases the activities of one group of lipogenic enzymes, *viz.*, FAS and CCE already responded to a significantly smaller amount of dietary sucrose than the other group of enzymes, *viz.*, ME, G6PDH and PGDH. Effective amounts of dietary sucrose intake were 1.0 *vs.* 1.8 g/100 g body weight/day for minimum and 3.7 *vs.* 6.8 g/100 g body weight/day for maximum stimulation in the first *vs.* the second group of enzymes, respectively.

All responses of the lipogenic activities to dietary manipulations occurred in a coordinate fashion, *i.e.* activities displayed the same frequency of oscillations and the same relative ratios of increase. Activities of FAS and CCE generally responded stronger and with higher sensitivity to

dietary manipulations than those of the other enzymes, suggesting a certain degree of independence with respect to their adaptive responses. Thus, the existence of (a) common obligatory step(s) for the general regulation of responses to dietary stimuli is likely, but additional factors or steps must exist which allow individual variations or fine-tuning of that common response to occur.

The activities of the lipogenic enzymes in liver and BAT form a reversible induction-repression system, which displays characteristic changes under specific conditions. These changes include synthesis and degradation of both mRNA and enzyme protein. Recent investigations using cRNA probes showed that changes of the lipogenic activities (FAS, acetyl-CoA carboxylase) in rat liver were paralleled by changes in the abundance of mRNA under various physiological conditions (Nepokroeff *et al.*, 1984; Pape *et al.*, 1988; Paulauskis and Sul, 1989). In avian liver the rate of synthesis of lipogenic enzymes was entirely dependent on the abundance of the respective mRNAs (Paulauskis and Sul, 1989). Dietary regulation plays a major role in this phenomenon (Perdereau *et al.*, 1990). Studies on the rate of transcription of the FAS gene suggested that dietary regulation operates primarily at the transcriptional level (Goodridge, 1987; Paulauskis and Sul, 1989).

Age dependence of the diet-induced variations of lipogenic enzyme activities

The dietary variations of the activities of the lipogenic enzymes of liver and BAT exhibited age-related differences. Induction of activity was high in young animals but decreased with age. Similarly the oscillations were more pronounced in young than in old animals. The age-related responses were similar in liver and in BAT. Diet-induced variations in old (250 days) animals were, on the average, only one-half to one-third of those observed in young (35 days) animals. However, the difference in the sensitivity of response of the two groups of lipogenic enzymes to changes in diet composition did not disappear during aging. The data suggest that in older animals which tend to have considerably more fat, the regulation of lipogenic activity looses much of its adaptive capacity, but not the sensitivity to respond to dietary stimuli.

Polychlorinated biphenyls and lipogenic enzyme activities

Effects of PCB feeding on the lipogenic enzyme activities in liver

As the PCB-induced effects did not display any sex dependence in liver (see, however, BAT), only data from male animals are shown. When animals, previously kept on the standard rodent chow, were switched to a PCB-containing diet, activity of the lipogenic enzymes increased. The activities responded again in two groups (FAS and CCE *vs.* ME, G6PDH and PGDH respectively) and only the responses of FAS and of G6PDH are displayed in Fig. 2. The PCB-induced increases were dose-dependent, showing little or no response below 30 ppm PCBs in the diet. With 60 ppm or more both groups of enzymes showed an increase in activity which began immediately in the FAS group (Fig. 2A), but only after 11 days of PCB feeding with the G6PDH group of enzymes (Fig. 2B). The response of the FAS group peaked at about twice the original level with 120 ppm PCBs in the diet at 4 days of feeding, but enzyme activities returned towards normal thereafter. At 250 ppm any response was abolished (Fig. 2A). Other investigations (Kling and Gamble, 1987) reported an inhibition of CCE activity with 100 ppm Arochlor 1254 in the diet. The G6PDH group displayed a slow increase over 10 days of PCB feeding, followed by a sharp rise thereafter, peaking with the highest dose, 250 ppm, at 20 days of feeding with a 10-fold induction (Fig. 2B). Similar to the response of enzyme activities in the FAS group those in the G6PDH group apparently returned towards normal immediately after peaking. All PCB-induced effects on the other lipogenic activities of the respective group (CCE; ME and PGDH) were of the same order of magnitude as shown for FAS and G6PDH, respectively.

Effect of PCBs on the starvation-refeeding response of lipogenic enzymes in liver

When previously starved animals were refed, activities of the lipogenic enzymes in liver increased strongly (Fig. 3, full symbols; see also Fig. 1). Addition of PCBs to the refeeding diet dose-dependently induced enzyme activities even higher (Fig. 3, open symbols). The additional increase was

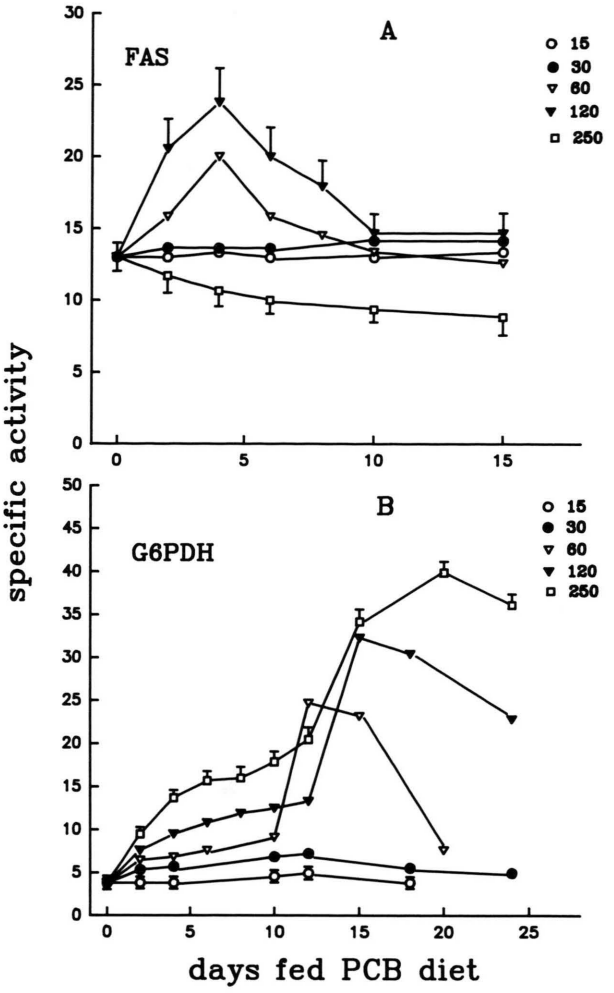

Fig. 2. The effect of PCB feeding on the activities of lipogenic enzymes in rat liver. Male rats were fed the balanced stock diet for 7 d and the PCB-containing stock diet beginning at time zero. Numbers indicate ppm levels of PCBs. Mean values of 4 individuals. SEM is shown in upper and lower curves only. Standard errors of intermediate curves are in a corresponding range.

already seen with 15 ppm PCBs and it was dose-dependent for FAS and CCE up to 60 ppm PCBs. Stimulation was increasingly abolished above this concentration. With the other enzyme activities, stimulation dose-dependently increased up to 250 ppm PCBs in the refeeding diet but it was completely abolished with 500 ppm PCBs, where the activities were lower than in the PCB-free controls, evidently demonstrating acute toxicity at this dose of PCBs (*cf.* Introduction). With FAS and CCE, maximum stimulation occurred after 2 days but was short-lived. After decreasing to near-normal levels enzyme activities in control and treated livers displayed similar oscillations (Fig. 3, top panels). The activities of ME and G6PDH (and PGDH, data not shown) displayed again a biphasic increase (*cf.* Fig. 2B) which peaked around day 6 (Fig. 3, bottom panels); beyond that time the activities declined again.

Oscillations of the lipogenic activities occurring in response to starvation-refeeding also occurred after starvation and refeeding a PCB-containing diet, but only with FAS and CCE. In the case of the other lipogenic enzymes such oscillations may begin after the 9th day of refeeding a PCB-containing diet, *i.e.* beyond the period of observation of this study. The two groups of enzymes responding with different sensitivity to dietary manipulation, *viz.* FAS and CCE on the one and ME, G6PDH and PGDH on the other hand were also observed when a PCB-containing diet was fed (Fig. 2). And a similar grouping of enzyme responses can be seen with γ-hexachlorocyclohexane (lindane): When starved animals were refed diets containing lindane, the activities of FAS and CCE were markedly inhibited, while the same amount of lindane in the diet induced the activities of the three other enzymes (Boll *et al.*, unpubl. data). The fact that ME, G6PDH and PGDH responded in a protracted fashion to PCB exposure may be explained by their dual role: not only do they provide reducing equivalents for lipogenesis, where FAS and CCE participate, but they also supply reducing equivalents to the xenobiotic metabolizing phase I enzymes of liver which are strongly induced by PCBs (Kato *et al.*, 1980). Consequently, this type of response was observed only in liver but not in BAT (see below).

Recovery after termination of PCB exposure

Discontinuation of feeding a PCB-contaminated diet resulted in additional, dose-dependent changes of the activities of the lipogenic enzymes in liver. Results obtained with ME as representative activity in the livers of female animals are shown in Fig. 4. Continued feeding of the contaminated diet beyond 12 days (Fig. 4, open symbols) resulted in essentially the same changes as shown in Fig. 2B. After 12 days of feeding a diet contain-

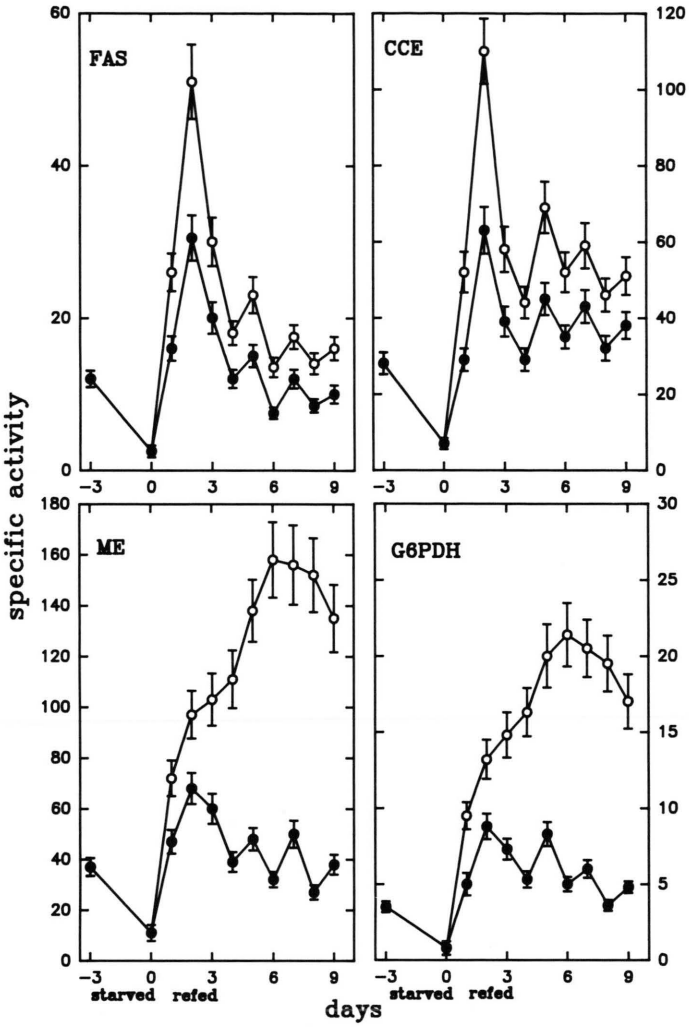

Fig. 3. The effect of PCBs on the starvation/refeeding response of lipogenic enzymes in rat liver. Female animals, previously fed the balanced stock diet were starved for 3 d and subsequently refed with the PCB-free balanced stock diet (full symbols) or with the stock diet containing 60 ppm PCBs (open symbols). Mean values of 4 individuals ± SEM.

ing 60 ppm PCBs ME activity was 2.5-fold increased, but it immediately returned to normal within a short time after discontinuation of feeding the PCB-containing diet (Fig. 4 A). With 120 ppm PCB in the diet, induction of the ME activity was 3.3-fold. Here induction was terminated and only after 8 days the enzyme activity returned to normal levels (Fig. 4 B). With 250 ppm in the diet, ME activity increased 4-fold within 12 days, but increased further when the PCB contamination was removed from the diet (Fig. 4 C). A dose-dependent induction of ME activity by TCDD has also been shown and the effect was attributed to the TCDD-induced reduction of thyroxine levels (Roth *et al.*, 1988). It is likely that the present findings are due to a comparable effect of the PCBs which also reduce thyroxine levels (Safe, 1984).

These results have considerable impact for the assessment of PCB exposure. At least with respect to the lipogenic enzyme activities, low level PCB exposure will have short-lived effects, whereas high level exposure may have quite persistent effects related to the toxicokinetics of PCBs. Chlorinated aromatic hydrocarbons induce their own metabolism (Safe, 1984), and this process is usually completed within a few days. Fig. 4 A indicates rapid metabolic removal of the PCBs with concomitant return of ME activity to normal. Fig. 4 B suggests that the xenobiotic-metabolizing enzyme systems had reached their limit of capacity and a

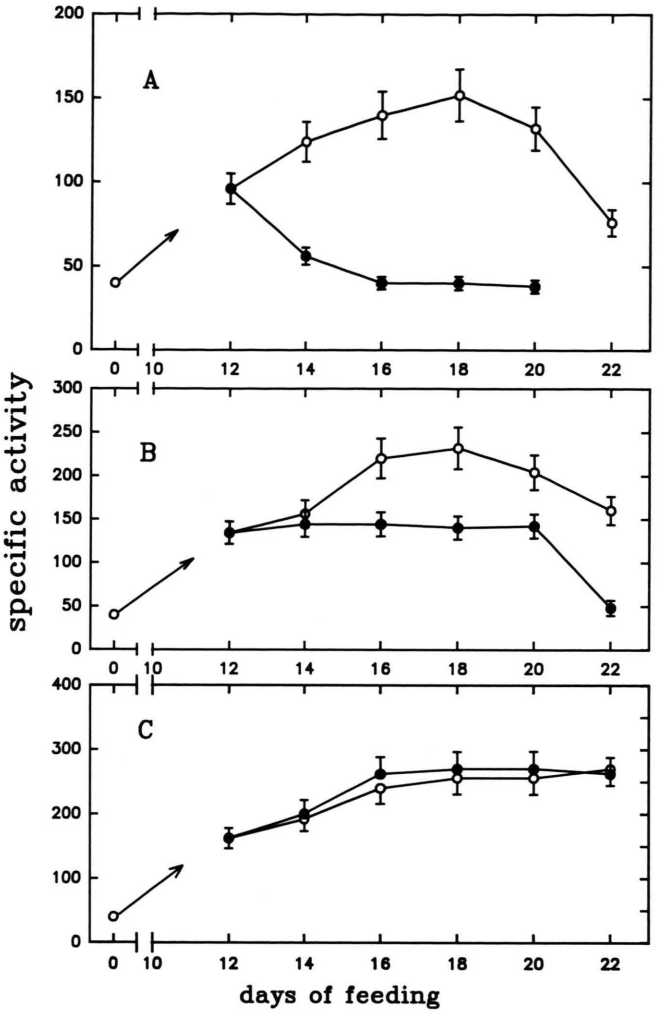

Fig. 4. Response of liver malic enzyme activity to termination of PCB feeding. Female animals were fed a balanced stock diet containing 60 ppm (A), 120 ppm (B), or 250 ppm (C) PCBs for 12 d and were then either switched back to the PCB-free balanced stock diet (full symbols), or continued on the respective PCB diet (open symbols). Values of 3–4 individuals ± SEM.

pool of PCBs was established in depot fat which took the enzymes about 8 days to degrade to a level where the activity of ME could return to normal. The situation represented in Fig. 4 C does not shown any signs of recovery of enzyme activity; however, it can be assumed that after a longer recovery period on the PCB-free diet the pool of PCBs would also decrease to a point where the activity of ME would return to normal.

Influence of age on the response to PCBs

The PCB-induced effects on the lipogenic activities decreased in magnitude with increasing age of the animals (Fig. 5). When feeding a diet containing 250 ppm the activities of ME, G6PDH and PGDH increased up to 4-fold in 35-day-old animals (Fig. 5, open symbols), but no more than 2-fold in 250-day-old animals during the 6-day period (Fig. 5, full symbols). The response of the activities of FAS and CCE to 250 ppm PCBs was abolished independent of age (Fig. 5; *cf.* Fig. 2 A).

Effect of PCBs on the lipogenic enzymes in brown adipose tissue

The activities of lipogenic enzymes in BAT responded to PCBs in a sex-specific manner (Fig. 6). When male rats were fed 200 ppm PCBs in the diet, activities of all five enzymes steadily de-

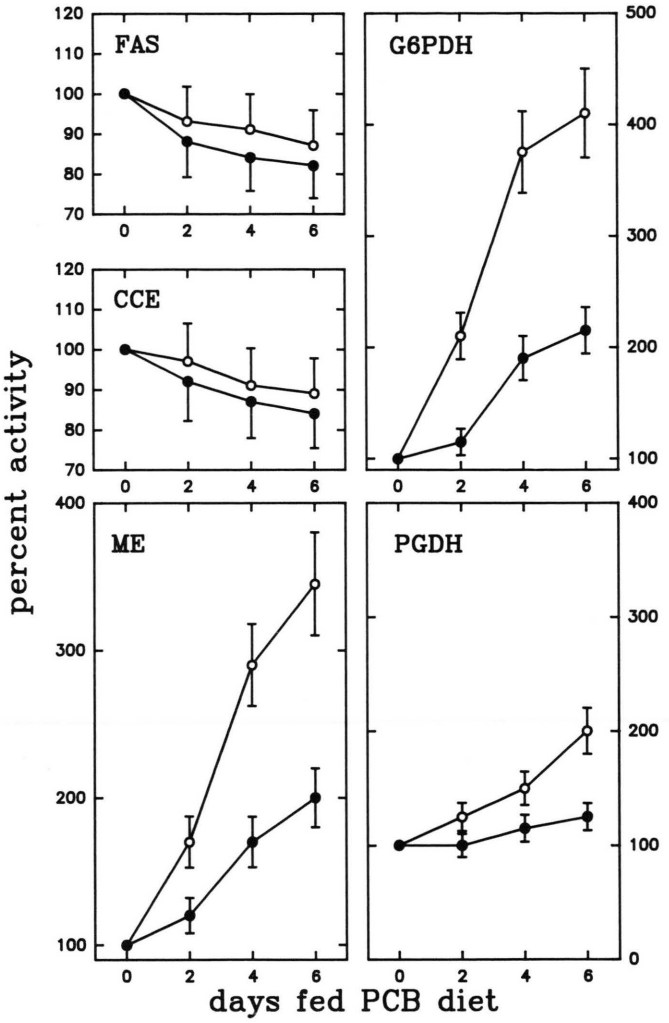

Fig. 5. Age-related responses of liver lipogenic enzymes to PCB feeding. Male rats were kept on the balanced stock diet for 7 d. PCB feeding (250 ppm) was initiated at time zero. Open symbols: 35 d old, full symbols: 250 d old animals. Data are percent of the specific activity of controls fed the PCB-free balanced stock diet. For absolute values of basal enzyme activities see Table II a, b. Means of 3 (250 d) or 4 (35 d) animals ± SEM.

creased over a 4-week period. In BAT of female rats 200 ppm PCB in the diet caused an increase of the activities of FAS, CCE and ME, and to a lesser extent of G6PDH and PGDH. These PCB-induced effects were dose-dependent in both sexes; at 30 ppm changes of enzyme activities were barely discernible and at 100 ppm they were significantly smaller than at 200 ppm (data not shown). As described for liver, the responses of the lipogenic activities in BAT towards PCB exposure were also blunted with increasing age (*cf.* Fig. 5).

TCDD exerts similar effects on lipogenesis as PCBs do, probably by an identical mechanism. The type of effects observed, however, depends on the type of assay, and possibly also on the strain of rats under investigation. Using the incorporation of [^{3}H]water into newly synthesized fatty acids an increase of hepatic, and a decrease of BAT lipogenesis was observed after a lethal dose of TCDD in male rats (Gorski *et al.*, 1988). With the [^{14}C]acetate method, using lower doses and a different strain of rats, a decrease was found in liver (Lakshman *et al.*, 1988). Finally, when acetyl-CoA carboxylase activity was studied, a strong inhibition of hepatic lipogenesis was seen (McKim *et al.*, 1991), but this obviously was due to lipid-induced direct inhibition of the enzymes as a result of the concomitant developing fatty liver. It cannot be excluded, that a similar effect is responsible for the decrease of FAS activity observed after 4 days (Fig. 2 A).

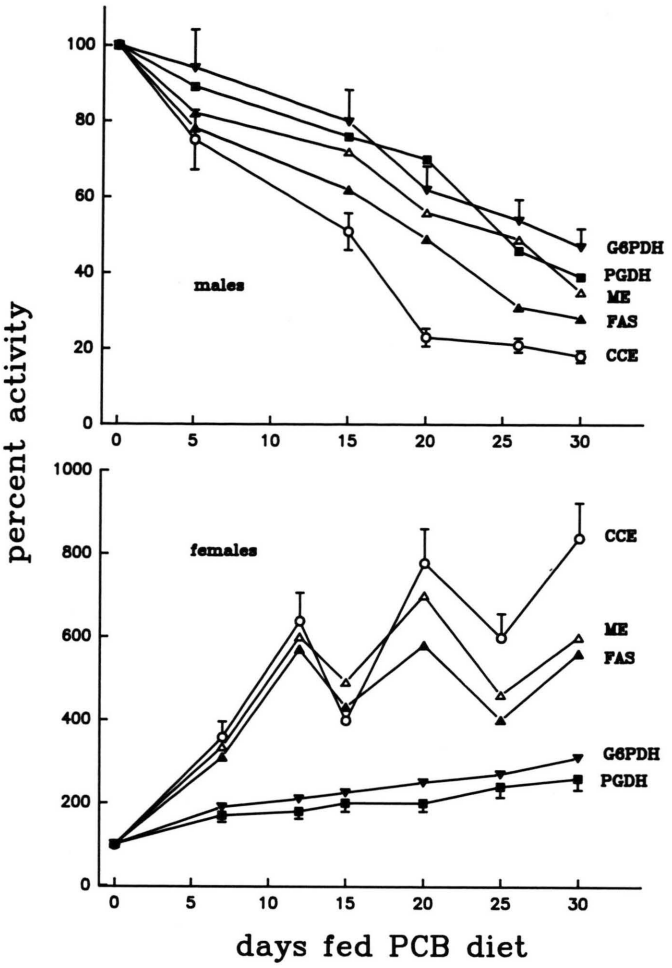

Fig. 6. Effect of PCBs on lipogenic enzymes in brown adipose tissue of male and female animals. Rats, 35 d old, were kept on the balanced stock diet for 7 d; PCB feeding (200 ppm) was initiated at time zero. Values are percent of specific activity in controls fed the PCB-free balanced stock diet. For absolute values of basal enzyme activities see Table IIe, g. Means of 3–4 animals. SEM is shown in upper and lower curves only. Standard errors of intermediate curves are in a corresponding range.

PCBs strongly reduce serum thyroxine levels (Safe, 1984); the same has been shown for TCDD, which also causes an increase of triiodothyronine levels (Gorski *et al.*, 1988). High circulating levels of triiodothyronine (rather than thyroxine) down-regulate lipogenesis in liver, but in BAT endogenous conversion of thyroxine to triiodothyronine *via* 5′-deiodinase is predominant. Thus, an increased rate of *de novo* fatty acid synthesis in liver with a concomitant decrease in BAT is to be expected with PCBs as well as with TCDD (Gorski *et al.*, 1988). Since *de novo* fatty acid synthesis was studied in male rats only (Gorski *et al.*, 1988), it is at this point not possible to speculate if differences in hormonal response and/or hormonal regulation are responsible for the different response of BAT to PCB exposure.

Effects of PCBs on serum lipids

PCB-induced changes of serum lipids have been described before (Yagi and Itokawa, 1980; Quasi *et al.*, 1984). Upon feeding diets containing PCBs, serum triglycerides, cholesterol and phospholipids all dose-dependently increased. For example, in the presence of 120 ppm dietary PCBs serum levels after 2 d of feeding increased 160, 40 and 50% for triglycerides, cholesterol and phospholipids, respectively. The effects were transient at the lowest PCB exposure (30 ppm in the diet), but (with the exception of triglycerides) persisted at elevated levels with the higher doses (data not shown). Although 250 ppm PCBs in the diet appeared to be inhibitory to FAS and CCE, the two enzymes directly involved in fatty acid synthesis

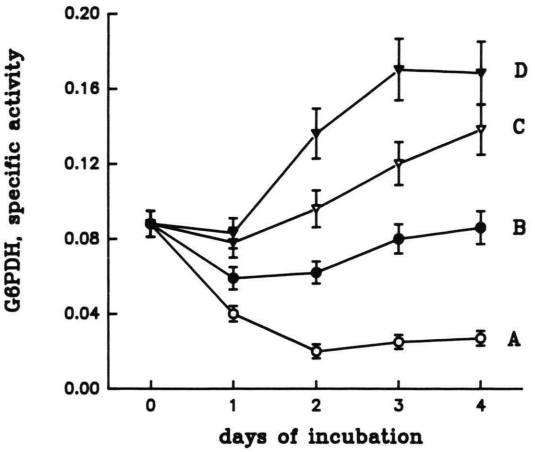

Fig. 7. Effect of PCBs on glucose-6-phosphate dehydrogenase activity of cultured rat hepatocytes. For media and details see Experimental section. A: basal (hormone-free) medium, B: basal medium plus 10 μM PCBs, C: induction (hormone-containing) medium, D: induction medium plus 10 μM PCBs. Mean ± SEM of 4 replicate incubations.

(see Fig. 2 A), triglyceride levels in the serum were still significantly increased with this PCB dose. Therefore, it appears that there is no immediate relation between changes of lipogenic enzyme activities in liver and the levels of the serum triglycerides. Serum lipid levels decrease during starvation and increase upon refeeding (Boll *et al.*, 1985; Schiller *et al.*, 1985). This increase was more pronounced when PCBs (15–250 ppm) were added to the refeeding diet, resulting in a maximum 3-fold elevation of the serum lipid values.

Effects of PCBs on the lipogenic enzymes of cultured hepatocytes

The activities of lipogenic enzymes in primary hepatocyte cultures all responded in a similar fashion to PCBs and G6PDH activity is shown representative for all 5 enzymes (Fig. 7 and 8). In basal (hormone-free) medium the activity of G6PDH decreased with time (Fig. 7 A), probably because of a lack of regulatory hormones in the culture medium. Addition of insuline and triiodothyronine not only prevented the loss of G6PDH activity, but resulted in a 2-fold stimulation after a lag period of 1 day (Fig. 7 C), indicating intact hormonal regulation. With 10 μM PCB present in the hormone-free medium decrease of G6PDH activity was significantly less and it was transient (compare A and B in Fig. 7). This could indicate a stimulating effect of the PCBs and would mimic the presence of a hormone. With insuline and triiodothyronine in the medium PCBs still had a stimulatory effect (compare C and D in Fig. 7). As the concentration of these hormones was at saturation levels for the respective receptors, an additional mechanism (receptor) for the PCB-induced effect must be involved. The precise nature of this mechanism (receptor) remains, however, unknown.

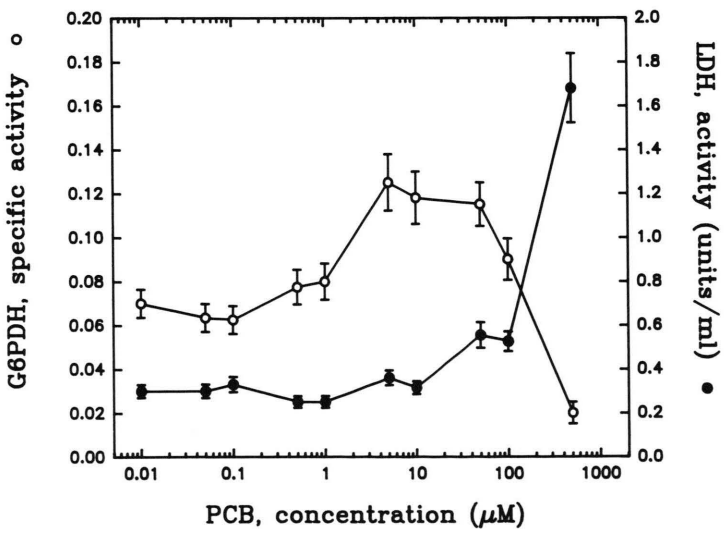

Fig. 8. PCB dose depencence of induction of glucose-6-phosphate dehydrogenase and leakage of lactate dehydrogenase in cultured rat hepatocytes. Activities of G6PDH and LDH were determined after 45 h of incubation of hepatocytes. Left ordinate: G6PDH, specific activity (open circles); right ordinate: LDH (determined in the medium), activity (units/ml) (closed circles). Values are means of 4 replicate incubations ± SEM.

Dose-dependence of the PCB-induced effects on the activity of G6PDH in hepatocytes was studied in Fig. 8 (open symbols). Leakage of LDH into the medium was determined as measure of toxicity (cell damage) (Fig. 8, closed symbols). The PCB-mediated induction of G6PDH activity began above 0.1 μM PCBs and was clearly dose-dependent up to about 10 μM PCBs, where the experiments of Fig. 7 were performed. Beyond a concentration of 100 μM the PCBs were overtly toxic, as evidenced by decreasing activity of G6PDH in the cells (Fig. 8, left ordinate) and increasing leakage of LDH into the culture medium (Fig. 8, right ordinate). The PCB-mediated induction of G6PDH was completely prevented by addition of 1μM cycloheximide to the medium. Thus, the PCB effect involved protein synthesis rather than activation of existing enzyme protein.

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