

The Effect of γ -Hexachlorocyclohexane (Lindane) on the Activities of Liver Lipogenic Enzymes and on Serum Lipids in Rats

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The effect of dietary γ -hexachlorocyclohexane (lindane) (50–350 ppm, 0.17–1.19 μ mol/kg chow) on the activity of enzymes of lipogenesis, viz., 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), and on serum lipid levels, was investigated in livers of 35-day-old male Wistar rats.

Lindane (150 ppm) caused a substantial decline of enzyme activities within the first 24 h of treatment. The decrease was transient, however, and enzyme activities subsequently recovered despite continuation of lindane feeding. The recovery of enzyme activities was comparatively fast in the case of ME, G6PDH and PGDH, but very slow with FAS and CCE.

Activities of lipogenic enzymes decrease when animals are starved, and increase much beyond prestarvation levels upon subsequent refeeding. Lindane in the refeeding diet blunted this overshoot of FAS and CCE activities in a dose-dependent manner. In contrast, activities of ME, G6PDH and PGDH responded to low dietary lindane concentrations with a substantial stimulation of the increase of activity, whereas at high lindane concentrations the overshoot was inhibited.

According to their responses to lindane exposure, liver lipogenic enzymes could be grouped into 2 categories with FAS and CCE representing one and ME, G6PDH and PGDH representing the other group.

Polychlorinated biphenyls (PCBs) in the diet caused basically opposite changes of the activities of the lipogenic enzymes. Co-administration of lindane and PCBs resulted in an apparent cancellation of effects, suggesting that lindane and PCBs affect fatty acid synthesis at opposite points.

Levels of the serum triglycerides were increased significantly as a result of lindane feeding, while serum cholesterol and phospholipid levels were only slightly elevated. The increase of serum triglyceride levels that is routinely observed after refeeding of starved animals was stimulated even more by low concentrations of lindane in the refeeding diet, but inhibited by high concentrations.

Introduction

Lindane (γ -hexachlorocyclohexane) is an important organochlorine pesticide intensively used for agricultural purposes, primarily in developing countries, but is also used in humans to treat infestations with lice or scabies. It is more toxic to insects than to vertebrates, but high-dose or prolonged exposure cause toxicity in mammals that is

usually associated with biochemical, functional and histological alterations of tissues such as testes, skin, kidney, liver and central nervous system. Chronic exposure causes hepatocarcinoma in mice, but not in rats. For a recent review on effects of lindane see Smith (1991).

Lindane is highly lipophilic; it does not accumulate specifically in any tissue or organ of the rat except storage fat. The distribution of the various hexachlorocyclohexane isomers is greatly influenced by metabolism (see Smith, 1991). Lindane is metabolized primarily in the liver *via* dehydrochlorination leading mostly to chlorophenols, or *via* dehydrogenation, dechlorination or hydroxylation.

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tion (see Smith, 1991). As biotransformation by any of the first three pathways is usually followed by hydroxylation, a variety of hydroxylated lindane metabolites is formed (Chadwick *et al.*, 1983). Since lindane induces cytochromes IIB1 and IIB2 (phenobarbital type), prolonged treatment accelerates elimination and shortens body half-life (see Smith, 1991). In rats whole body half-lives between 1 and 10 weeks were reported, depending on the status of cytochrome induction. The time to reach dispositional equilibrium with continuous exposure depends on the level of exposure; at 100 ppm lindane in the feed, about 1 week was required to attain equilibrium of intake and excretion in the rat (see Smith, 1991).

In a previous study it was demonstrated that the highly lipophilic polychlorinated biphenyls (PCBs) affect the activities of lipogenic enzymes in the liver of rats (Boll *et al.*, 1994). It was of interest to investigate, if lindane or a combination of lindane and PCBs exert similar effects on lipogenesis as the PCBs do. Despite its tendency to accumulate in storage fat, the organ most sensitive to lindane is liver, where it can cause marked toxicity (see Smith, 1991). The synthesis of fatty acids is a reaction sequence of major physiological importance; any agent that interferes with lipid homeostasis might result in redistribution of lipophilic environmental pollutants associated with storage fat, and ultimately shift or affect organ toxicity.

Experimental

Animals and diets

Wistar rats from our own breeding station were used and maintained in a constant light/dark cycle (lights on: 6 a.m. to 6 p.m.) in a humidity-controlled room at $25 \pm 2^\circ\text{C}$. Male animals of 120 g body weight (35 days old) were used throughout the study. They were kept on a balanced stock diet (standard diet tpf 1324; Altromin, D-49828 Lage, Germany). For its composition see Boll *et al.* (1994). Except under fasting conditions the animals had free access to feed.

Lindane- or lindane plus PCB-containing diets were prepared by slowly adding an ethanolic solution of the pollutant(s) under stirring to the powdered stock diet (50 ml ethanol per 500 g diet). The mixture was then pasted with water, pellets were formed and dried at 25°C for 3–4 days. Con-

trol animals received an identical diet prepared with ethanol alone. This diet is designated as lindane-free balanced stock diet. For the feeding experiments with lindane, animals were kept on the standard diet (see above) for 7 days prior to the initiation of lindane feeding. Likewise, animals used for the starvation/refeeding experiments were held for 7 d under controlled feeding conditions with the standard diet before starvation was initiated. Maximum lindane concentrations in the diet were 350 ppm; feeding diets with 500 ppm or more lindane resulted in decreased feed intake and body weight gain, and in a generally poor health status of the animals.

Tissue sampling and preparation of crude extracts

Livers were removed between 8 and 9 a.m. from ether-anesthetized animals, and rapidly cooled 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). Aliquots were homogenized in the above medium (1 g tissue/3 ml medium) in a Potter-Elvehjem homogenizer with 20 strokes. Liver homogenates were centrifuged at $20,000 \times g$ for 30 min, the resulting supernatant centrifuged at $105,000 \times g$ for 60 min and the resulting cytosols used as the enzyme source.

Enzyme assays

The lipogenic enzymes were assayed spectrophotometrically as described elsewhere: FAS (Hsu *et al.*, 1963), CCE (Inoue *et al.*, 1966), ME (Hsu and Lardy, 1967), G6PDH (Löhr and Waller, 1974) and PGDH (King, 1974). Specific activities are expressed as $\mu\text{mol}/\text{min} \cdot \text{mg}$ protein at 25°C . Protein was determined with the method of Lowry *et al.* (1951) using bovine serum albumin as the standard.

Serum lipids

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

Chemicals

Lindane (γ -hexachlorocyclohexane) and all biochemicals were obtained from Sigma Chemical Company, St. Louis, U.S.A. (D-82041 Deisenhofen). Clophen A-50 (source of polychlorinated biphenyls) was a product of Bayer AG, Leverkusen, Germany. For composition of the PCB mixture see Boll *et al.* (1994).

Results and Discussion

Liver lipogenic enzymes and serum lipids

Specific activities of the investigated enzymes of lipogenesis were determined in livers of rats (see above), kept on standard rodent chow. The following basal activities were found ($\mu\text{mol}/\text{min} \cdot \text{mg}$ protein, mean \pm S.D., $n = 4$): FAS, 12.3 ± 1.2 ; CCE, 25.4 ± 2.3 ; ME, 45.7 ± 4.6 ; G6PDH, 3.8 ± 0.34 ; PGDH, 8.3 ± 0.6 .

In a previous study it was demonstrated that these basal activities are subject to major changes in response to the amount and type of feed intake (Boll *et al.*, 1994). The activities of all lipogenic enzymes decreased on starvation, and increased far beyond the prestarvation levels when the fasted animals were refed with diets of different composition. The increase was followed by pronounced oscillations of the activities, with magnitude and duration of the oscillations depending on the composition (*i.e.*, contents of carbohydrate and fat, respectively) of the refeeding diet (Boll *et al.*, 1994). The responses to dietary stimuli of the activities of FAS and CCE were similar; they occurred soon after relatively small changes of diet composition. The pattern of changes of the activities of ME, G6PDH and PGDH, on the other hand, was also uniform, but occurred more slowly and at higher doses than that of the former two enzyme activities.

Levels of serum lipids as determined in animals fed the standard rodent chow were (mg/ml serum): triglycerides 0.9 ± 0.10 ; cholesterol 0.75 ± 0.07 ; and phospholipids 1.3 ± 0.14 . Serum lipids decreased on starvation and increased on subsequent refeeding (Boll *et al.*, 1985; Schiller *et al.*, 1985) (see also Fig. 5 A and B, full symbols). The refeeding-induced increase depended on the composition of the refeeding diet, *i.e.* it was greater with a high-carbohydrate, fat-free diet than with the standard-

balanced diet (Boll *et al.*, 1985). Serum lipid variations were significantly more pronounced in young (35 d) than in older (150 d) animals (Boll *et al.*, 1985). In response to refeeding triglyceride levels increased immediately while those of cholesterol and phospholipids only after 1 day began to increase (Boll *et al.*, 1985) (compare Fig. 5 A and B, full symbols). Changes of the serum lipids in response to these metabolic variations were reflected in the lipoprotein profiles (Boll *et al.*, 1985).

Effect of feeding a lindane-containing diet on the activities of liver lipogenic enzymes

Replacing the standard lab chow by a diet containing 150 ppm lindane caused a precipitous drop of the activities of all 5 enzymes within 24 h (Fig. 1). The activities of FAS and CCE were most substantially affected, decreasing 60 to 75%. The decrease was dose-dependent (data not shown). The activities of the other three enzymes were not as strongly affected, but also responded in a dose-dependent manner. Despite continued feeding with lindane, all enzyme activities began to re-

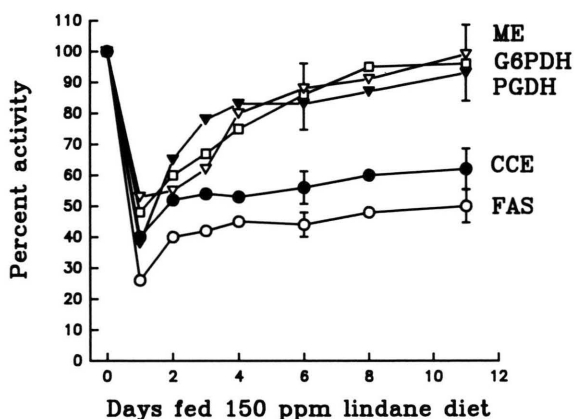


Fig. 1. The effect of lindane feeding on the activity of liver lipogenic enzymes. Rats were fed the balanced stock chow for 7 d and the lindane-containing diet (150 ppm) beginning at time zero. Values are percent of basal activity (control animals receiving the lindane-free balanced stock diet). For absolute values of basal enzyme activities see text. For reasons of clarity SEM is shown at two points only, standard errors in other curves are in a corresponding range. Mean values of 4 individuals. ME, malic enzyme; G6PDH, glucose-6-phosphate dehydrogenase; PGDH, phosphogluconate dehydrogenase; CCE, citrate cleavage enzyme; FAS, fatty acid synthase.

cover immediately after the initial decline (Fig. 1). FAS and CCE activities increased very slowly, reaching only about 50% of control (pre-lindane feeding) levels by 11 days after the onset of lindane feeding. The activities of ME, G6PDH, and PGDH recovered at a fast pace for 3 days, then slowed down somewhat, but reached almost control levels by day 11 (Fig. 1). Peaking of enzyme activities after lindane administration with subsequent return to normal levels despite continued feeding of the pesticide has also been observed in livers of mice, but no mechanism of action was suggested for this finding (see Smith, 1991).

The activities of the same five lipogenic enzymes in liver responded in an essentially opposite manner to feeding a diet that contained PCBs (Boll *et al.*, 1994). Yet, it has to be emphasized that in either case the activities of these enzymes responded in groups to the insult, *i.e.*, FAS and CCE together displayed one type and ME, G6PDH, and PGDH jointly displayed the other type of response (see Boll *et al.*, 1994).

Effect of lindane on the starvation-refeeding response of lipogenic enzyme activities

When animals were starved for 3 days, activities of lipogenic enzymes in liver decreased. The decline was more articulate with FAS and CCE (75–85%) than with the other enzymes (50–60%). Upon refeeding the stock diet the activities increased dramatically (Fig. 2, full symbols; *cf.* Boll *et al.*, 1994). This increase was followed by pronounced oscillations of the activities. The initial peak of enzyme activity of the starvation/refeeding response was between 48 and 72 h after onset of refeeding, varying from one experiment to another (compare Fig. 2 and Boll *et al.*, 1994). Addition of lindane to the refeeding diet again resulted in similar responses of the activities of FAS and CCE on the one hand, and of ME, G6PDH, and PGDH on the other hand. Therefore, only the changes of the activities of FAS (Fig. 2A) and G6PDH (Fig. 2B) are shown to exemplify the respective responses. Lindane in the refeeding diet blunted this response for FAS and CCE dose-dependently (Fig. 2A). With the highest, near-toxic dose of lindane, 350 ppm, no oscillations of activity were observed. In the case of ME, G6PDH, and PGDH the overshoot of enzyme activity after fasting and

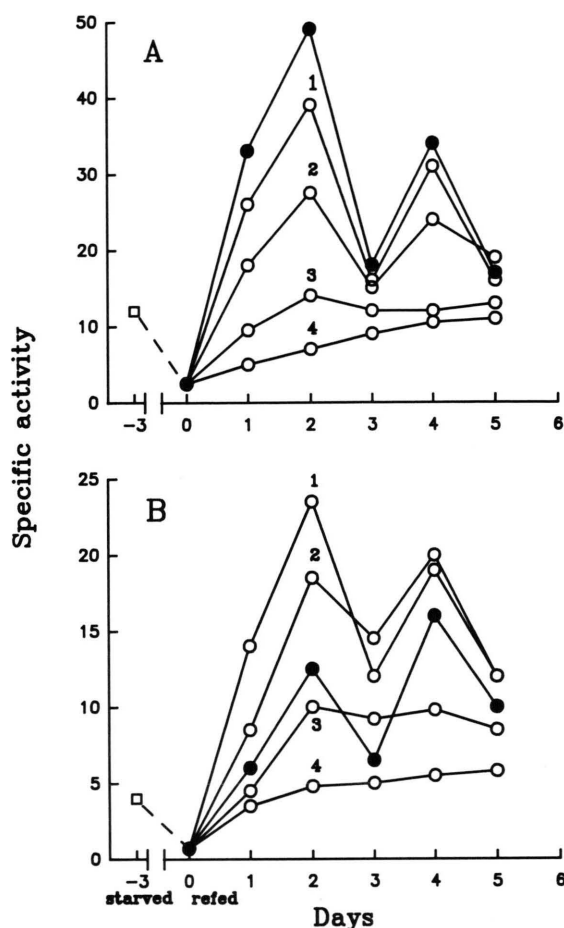


Fig. 2. The effect of lindane on the starvation/refeeding response of liver lipogenic enzymes. Panel A: FAS; panel B: G6PDH. Animals were starved for 3 d and subsequently refeed with the lindane-free balanced stock diet (closed symbols) or with the stock diet containing different concentrations of lindane (open symbols). Numbers indicate dietary lindane concentrations: (1) 50 ppm (0.17 $\mu\text{mol/kg}$ chow); (2) 150 ppm; (3) 250 ppm; (4) 350 ppm. Mean values of 4 individuals. Standard errors (6–13%) omitted for clarity.

subsequent refeeding the lindane-containing diet was even more pronounced than with refeeding the stock diet (Fig. 2B). This increased overshoot was also attenuated dose-dependently. While activity was still elevated at 200 ppm lindane, it fell below control levels at 250 ppm lindane and showed no more overshoot at the highest dose. With both groups of enzymes the refeeding-induced oscillations of activities were essentially abolished with and above the second highest dose

of lindane (250 ppm). Later during refeeding the activities of the enzymes tended to return to the control levels despite continued administration of the lindane-containing diet. Treatment with PCBs in the diet after 3 days of starvation caused similar reactions of the activities of FAS and CCE, but those of ME, G6PDH, and PGDH responded with a much stronger, biphasic overshoot and without any subsequent oscillations (Boll *et al.*, 1994).

Combined effect of lindane and polychlorinated biphenyls on the starvation-refeeding response of lipogenic enzyme activities

Fig. 3 summarizes the changes of the lipogenic enzymes in response to lindane and PCBs when both contaminants were present in the diet. Again the exemplary responses of FAS (Fig. 3A) and of G6PDH (Fig. 3B) are shown. 150 ppm lindane in the diet inhibited the refeeding-induced increase of FAS (compare curves 1 and 2), whereas 75 ppm PCBs stimulated the increase (curves 1 and 3) (*cf.* Boll *et al.*, 1994). With both substances present in the diet the increase of FAS activity was intermediate between the effects of each single compound (curve 4).

The response of the other group of enzymes, as exemplified by the activity of G6PDH (Fig. 3B), was a stimulation by both 150 ppm lindane (compare curves 1 and 2) and also by 75 ppm PCBs (curve 1 and 3) (*cf.* Boll *et al.*, 1994). With both contaminants present the initial increase of activity was approximately the sum of the 2 individual stimulations (see values for 1 d of refeeding). In refeeding diets containing PCBs, increase of the enzymes of the G6PDH group does not exhibit oscillatory changes as do the activities of the FAS group but they increase continuously (compare curves 3 in Fig. 3A and B) (see also Boll *et al.*, 1994). Consequently the further changes of the activity of G6PDH in the presence of the 2 contaminants (curve 4) are intermediate between lindane (curve 2) and PCBs (curve 3).

It has been suggested that due to its highly lipophilic character, lindane causes toxicity by impairment of essential membrane functions, as it accumulates in functional membranes of cellular organelles (Antunes-Madeira and Madeira, 1985). Although it does not influence general membrane

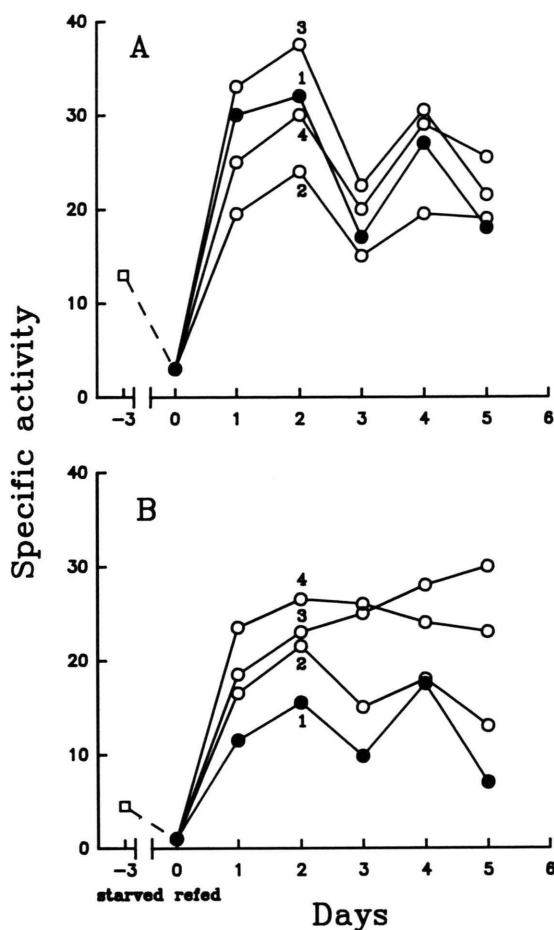


Fig. 3. Combined effect of lindane and polychlorinated biphenyls (PCBs) on liver lipogenic enzymes under conditions of starvation/refeeding. Panel A: FAS; panel B: G6PDH. Animals were starved for 3 d and subsequently refeed with the lindane-free balanced stock diet (1) (closed symbols) or with the stock diet containing either 150 ppm lindane ($0.51 \mu\text{mol/kg}$ chow) (2), 75 ppm PCBs ($0.22 \mu\text{mol/kg}$ chow) (3) or with 150 ppm lindane plus 75 ppm PCBs (4) (open symbols). Mean values of 4 individuals. Standard errors (6–13%) omitted for clarity.

fluidity (Antunes-Madeira and Madeira, 1989), it might still disturb ordered lipid domains surrounding integral proteins in the membrane, thus affecting the activity of certain membrane-bound proteins in a non-specific manner (Parries and Hokin-Neaverson, 1985; Jones *et al.*, 1985; Demaël *et al.*, 1987). In the case of the present study, a mechanism like this would have to act *via* membrane-associated receptors that relate extracellular, *e.g.*,

hormonal, messages to the cellular DNA. A chemical similarity between lindane and inositol has been pointed out, and it was suggested that lindane might interfere with processes that depend on phosphatidyl inositol (see Smith, 1991).

An alternative mechanism of action of lindane-induced toxicity could be related to the development of an oxidative stress condition, *i.e.*, the generation of oxygen-free radicals by microsomal enzymes (Junqueira *et al.*, 1986; Junqueira *et al.*, 1988). Lindane induces amount and activities of several cytochrome P-450 isoenzymes (Videla *et al.*, 1988) and of the activity of NADPH-cytochrome P-450 reductase (Srinivasan and Rhadkrishnamurty, 1983). In addition, lindane causes increased NADPH-dependent oxygen uptake of liver tissue together with increased rates of superoxide radical generation and lipid peroxidation (Junqueira *et al.*, 1988; Videla *et al.*, 1988). These biochemical changes were found to correlate with morphological lesions induced in the liver by lindane (Junqueira *et al.*, 1988). At the same time, intoxication with lindane induced a derangement of antioxidant mechanisms in the cells, *viz.*, decreased superoxide dismutase and catalase activities and changes in the glutathione status (Videla *et al.*, 1988; Barros *et al.*, 1988).

Effect of lindane on serum lipids

When animals on the standard rodent chow were fed a 150 ppm lindane-containing diet the levels of the serum triglycerides increased significantly (Fig. 4). Levels reached a maximum after

2 days of lindane feeding, declined for two days, and then began to rise again. The second increase led to a level of 60–70% above control. The time course of triglyceride levels was essentially inverse to that of the activities of lipogenic enzymes in liver in response to lindane feeding (Fig. 1). This incites to speculate that lindane caused a transient, strong mobilization of storage fat that led to increased serum triglyceride levels and to a feedback inhibition of the activities of lipogenic enzymes. Cholesterol and phospholipid levels, however, were not affected as they did not change significantly in response to lindane feeding: after 11 days of feeding the 150 ppm lindane diet levels were only 15–20% higher than in stock diet-fed controls (Fig. 4).

Changes of serum lipid levels in response to starvation and refeeding, and the effects of lindane on this response, are summarized in Fig. 5. The refeeding-induced increase which was transient for the triglycerides was even more pronounced when the refeeding diet contained 100–200 ppm lindane, but was blunted with 250 and 350 ppm lindane (Fig. 5 A, open symbols). This time course resembles the time course of G6PDH shown in Fig. 3 B and might indicate that the activities of the enzymes which provide reducing equivalents for lipogenesis (*viz.*, G6PDH, ME and PGDH) control the level of serum triglycerides under the given treatment conditions. A comparison of Fig. 1 and 4 vs. 2 and 5 emphasizes that the type of control of the activities of the 5 lipogenic enzymes differs greatly between adding lindane to the diet under normal feeding conditions, and a regimen

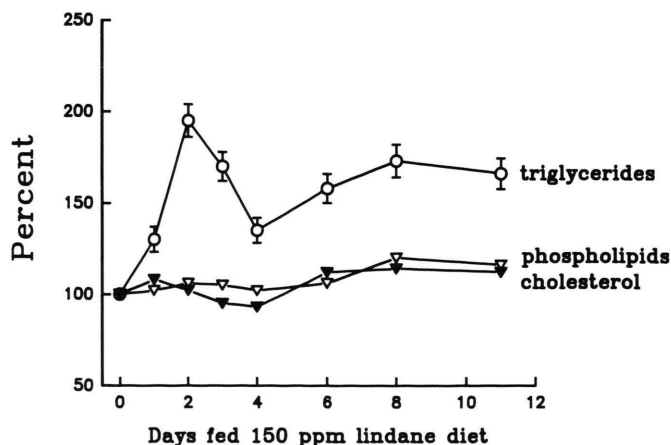


Fig. 4. Effect of lindane feeding on serum lipids. Animals were kept on the balanced stock diet for 7 d, feeding the lindane-containing diet (150 ppm) began at time zero. Data are percent of basal serum levels (control animals fed the lindane-free stock diet). For absolute basal values of serum lipids see text. Means of 4 animals. Standard errors shown for triglycerides only, errors in the other curves are in a corresponding range.

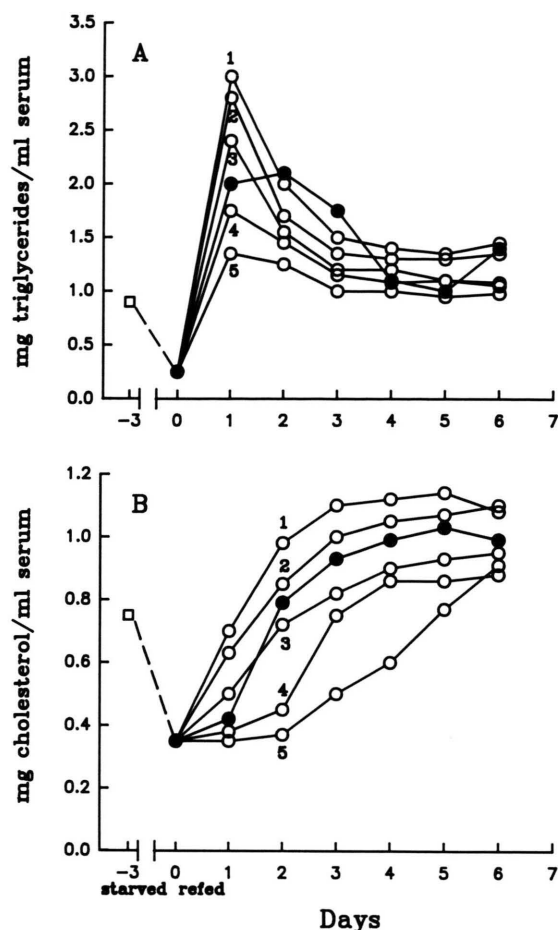


Fig. 5. Effect of lindane on the starvation/refeeding response of serum lipids. Panel A: triglycerides; panel B: cholesterol. Animals were starved for 3 d and subsequently refeed with the lindane-free stock diet (closed symbols) or with the stock diet containing different concentrations of lindane (open symbols). Numbers indicate dietary lindane concentrations: (1) 100 ppm ($0.34 \mu\text{mol/kg}$ chow); (2) 150 ppm; (3) 200 ppm; (4) 250 ppm; (5) 350 ppm. Values are means of 4 individuals. Standard errors (7–12%) omitted for clarity.

involving first starvation and then refeeding a diet with varying contents of lindane. The enzyme activities of Fig. 2A and 2B, as compared to the serum triglycerides in Fig. 5A over the same period of time, *viz.* 5 days, also suggest that the coupling of serum triglyceride levels to the activities of lipogenic enzymes is rather indirect, as the

oscillations of enzyme activities were not reflected in serum triglyceride levels.

Serum cholesterol decreased during starvation, increased with refeeding of the stock diet over 3 days, and remained elevated throughout the remainder of the 6 day investigation period (Fig. 5B). Changes of serum phospholipids always paralleled those of serum cholesterol (Boll *et al.*, 1985). The increase of serum cholesterol after refeeding starved animals was stimulated by low concentrations of lindane in the diet, but inhibited by higher concentrations (Fig. 5B). After 6 days of refeeding lindane-containing diets, however, serum cholesterol levels differed little between controls and treated animals. Changes of cholesterol and also phospholipid levels (data not shown) were significantly slower than those of the serum triglycerides (Fig. 5, *cf.* Boll *et al.*, 1985). The findings suggest that starving rats and then refeeding a diet contaminated with lindane triggers a complicated series of events that involves a major redistribution of lipids. The similarity in the dose responses of serum triglyceride and cholesterol levels also imply that the transport vehicles of triglycerides and cholesterol, *viz.*, the lipoproteins, are involved in the process.

Refeeding rats with PCB-containing diets had a seemingly similar effect on all three categories of plasma lipids, but with an inverse dose-response relationship (Boll *et al.*, 1994): Low doses of PCBs caused very little change of lipid levels, as compared to starved/refed controls, whereas high doses boosted lipid levels much above control values (Boll *et al.*, 1994). Administration of a diet contaminated with both lindane (150 ppm) and PCBs (75 ppm) to previously starved rats affected serum lipid levels basically in the same way as the activities of lipogenic enzymes (see Fig. 3), *i.e.*, little deviation from the levels in starved/refed control animals (data not shown). This, together with the data shown in Fig. 3, suggests that lindane and PCBs, respectively, affect lipogenesis at opposite points, and that administration of a combination of both pollutants can result in a no-effect situation, although the actions of both compounds might rather be additive for other endpoints of toxicity.

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