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INFLUENCE OF HYPERTHYROIDISM ON LINDANE-INDUCED HEPATOTOXICITY IN THE RAT

LUIS A. VIDELA,*† GLADYS SMOK‡ PILAR TRONCOSO,* KARIN A. SIMON,§ VIRGINIA B. C. JUNQUEIRA,§ and VIRGINIA FERNÁNDEZ*

Departamentos de Bioquímica, *Facultad de Medicina, Universidad de Chile, Santiago, Chile, and §Instituto de Química, Universidade de São Paulo, São Paulo, Brasil; and ‡Departamento de Anatomía Patológica, Facultad de Medicina, Universidad de Chile, Santiago, Chile

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Abstract—Parameters related to hepatic oxidative stress, cell injury, phagocytic activity, and liver histology were studied in control rats and in animals subjected to L-3,3',5-triiodothyronine (T_3) and/or lindane administration. Hyperthyroidism elicited a calorigenic response and increased rates of hepatic O_2 uptake, which were not modified by lindane treatment. T_3 diminished serum lindane levels as well as those in the liver and adipose tissue, whereas lindane enhanced serum T_3 levels in animals given T_3. Compared with control rats, lindane significantly increased the rate of formation of thiobarbituric acid reactants (TBARS) by the liver, with no changes in either the reduced glutathione (GSH) content, the TBARS/GSH ratio as indicator of oxidative stress, or in the fractional rates of lactate dehydrogenase (LDH) and GSH efflux from perfused livers as integrity parameters. Hyperthyroidism induced GSH depletion in the liver, with a significant enhancement in the TBARS formation, the TBARS/GSH ratio, and in the fractional LDH and GSH efflux. These parameters were increased further by joint T_3 and lindane administration in a magnitude exceeding the sum of the effects produced by the separate treatments. In addition, hyperthyroidism led to Kupffer cell hyperplasia and significant increases in serum glutamate oxalacetate transaminase (GOT) and in hepatic zymosan-induced chemiluminescence, while liver myeloperoxidase (MPO) activity was found unchanged, compared with controls. Rats treated with lindane presented normal liver histology, with no changes in biochemical parameters related to cell injury. The joint administration of T_3 and lindane, however, elicited a marked elevation in serum GOT and glutamate pyruvate transaminase (GPT), concomitantly with extensive liver necrosis and the presence of granulomas containing lymphocytes, Kupffer cells and polymorphonuclear leukocytes (PMN). In this condition, hepatic zymosan-induced light emission and MPO activity were enhanced over control values. It is concluded that hyperthyroidism increases the susceptibility of the liver to the toxic effects of lindane, which seems to be accomplished by potentiation of the hepatic oxidative stress status. The latter effect may be conditioned by an enhanced phagocytic respiratory burst activity due to the observed Kupffer cell hyperplasia and PMN infiltration, in addition to the increased production of reactive oxygen species in parenchymal cells.

Key words: hyperthyroidism; lindane; oxidative stress; liver damage; lipid peroxidation; Kupffer cell hyperplasia

The understanding of the mechanisms involved in the genesis of lethal cell injury has been a major concern of biochemical pathology. In the liver, the toxicity of xenobiotics is usually multifactorial, as shown for either carbon tetrachloride [1], acetaminophen [2], or ethanol [3], whose interaction potentiates hepatotoxicity [4].

The development of an oxidative stress condition in the liver by lindane (γ-hexachlorocyclohexane) intoxication has been considered as a possible hepatotoxic mechanism of the insecticide [5]. Lindane is biotransformed by liver microsomal enzymes to a variety of metabolites, including polychlorocyclohexenes that are susceptible to GSH conjugation for elimination [5]. In addition, induction of the liver microsomal cytochrome P450 system is also observed, with an enhancement in the rate of $O_2^{\cdot-}$[‖] generation [6] and in the magnitude of lipid peroxidation indicators [6–8]. Concomitantly, lindane alters some antioxidant mechanisms of the hepatocyte, including decreased superoxide dismutase and catalase activities [6]. The changes in these biochemical parameters related to oxidative stress are dose [9] and time [6] dependent, and coincide with the onset and progression of morphological lesions [6]. Due to the extensive use of lindane for public health purposes in developing countries and the incidence of toxicity in humans upon its exposure [10], it was considered important to investigate whether lindane-induced hepatotoxicity could be modulated by an additional source of oxidative stress. For this purpose, hyperthyroidism was chosen as an example of a condition involving an oxidant stress, evidenced in both experimental animals and humans [11]. This hormone dysfunction leads to an enhanced free radical activity in the liver associated with thyroid calorigenesis, with significant increments in the rates of $O_2^{\cdot-}$ and/or H_2O_2 production at microsomal [12] and mitochondrial [13] sites. In this work, parameters related to oxidative stress were measured in the liver of rats made hyperthyroid by treatment with T_3 and subjected to acute lindane intoxication, in relation to changes in serum transaminases and structural alterations in the liver parenchyma. In addition, the possibility that T_3 and/or lindane administration could condition an increased phagocytic activity in the liver

† Corresponding author: Dr. Luis A. Videla, Departamento de Bioquímica, Facultad de Medicina, Universidad de Chile, Casilla 70086, Santiago-7, Chile. FAX 56-2-7355580.

‖ Abbreviations: $O_2^{\cdot-}$, superoxide radical; T_3, L-3,3',5-triiodothyronine; PMN, polymorphonuclear leukocytes; GSH, reduced glutathione; TBARS, thiobarbituric acid reactants; LDH, lactate dehydrogenase; GOT, glutamate oxalacetate transaminase (aspartate aminotransferase); GPT, glutamate pyruvate transaminase (alanine aminotransferase); and MPO, myeloperoxidase.

was also studied. Different toxic conditions in the liver release chemoattractants and activating factors, leading to infiltration of PMN into the liver [14–16]. Activation of PMN infiltrating the liver and its resident macrophages, the Kupffer cells, produces large quantities of several reactive oxygen species [17, 18], which could constitute an additional source of oxidative stress.

MATERIALS AND METHODS

Experimental groups

Male Sprague-Dawley rats fed *ad lib.* received daily i.p. injections of 0.1 mg T_3/kg body weight or equivalent volumes of 0.1 N NaOH (T_3 diluent) for 3 consecutive days. At the third day of hormone treatment, animals were given either 20 mg of lindane/kg or equivalent volumes of lindane vehicle (corn oil) i.p., forming four experimental groups: (a) control–control [CC], (b) control–lindane [CL], (c) T_3–control [T_3C], and (d) T_3-lindane [T_3L]. Determinations were carried out 24 hr after the last treatment in groups of animals exhibiting comparable values of body weight [CC, 243 ± 12 g (N = 22); CL, 228 ± 12 (N = 22); T_3C, 235 ± 13 (n = 22); T_3L, 227 ± 15 (N = 22)] and liver weight/body weight ratios [CC, 3.77 ± 0.12 g/100 g (N = 22); CL, 4.06 ± 0.15 (N = 22); T_3C, 3.83 ± 0.12 (N = 22); T_3L, 3.93 ± 0.45 (N = 22)]. At this experimental time, serum T_3 levels were measured by the GammaCoat™ [^{125}I]T_3 Radioimmunoassay Kit (Baxter Healthcare Corp., Cambridge, MA), with the rectal temperature of the animals measured with a thermocouple, and the rate of O_2 consumption measured polarographically in perfused livers as described below. Also, lindane levels in serum, liver, and adipose tissue were determined by gas chromatography, as previously described [8].

Determination of GSH levels and TBARS formation

Groups of animals were anesthetized with Nembutal (50 mg/kg, i.p.) and the livers were perfused *in situ* with 200 mL of a cold solution containing 140 mM KCl and 10 mM potassium phosphate buffer, pH 7.4, to remove blood. Hepatic GSH levels were determined in liver homogenates (1:100) by the catalytic assay of Tietze [19]. TBARS formation was assayed in liver homogenates (1:4) prepared in 140 mM KCl containing 10 mM potassium phosphate buffer, pH 7.4, after centrifugation at 900 *g* for 20 min at 4° and incubation for 30 min at 37° [6]. Results are expressed as nanomoles per milligram protein per hour. The protein content of liver supernatants was measured according to Lowry *et al.* [20].

Liver perfusion and sinusoidal GSH and LDH efflux

Livers obtained from rats under Nembutal (50 mg/kg, i.p.) anesthesia were perfused with a solution containing 118 mM NaCl 4.8 mM KCl, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 2.5 mM $CaCl_2$, 25 mM $NaHCO_3$, and 10 mM glucose, equilibrated with an O_2:CO_2 mixture (19:1, v/v) to give pH 7.4, at constant flow rates (3.0 to 3.8 mL/g liver/min). Perfusions were carried out at 36–37° without recirculation of the perfusate. Oxygen consumption was determined polarographically [7] in the effluent perfusate collected via a cannula placed in the vena cava and allowed to flow past a Clark-type oxygen electrode. Livers were allowed to equilibrate for 15 min, and six perfusate samples were taken every 5 min for the determination of GSH concentration [19] and LDH activity [21] (one unit of LDH activity represents 1 μmol/min at 25°). The mean of the six determinations and the respective perfusion rate were employed to calculate the rate of GSH (nanomoles per gram liver per minute) and LDH (milliunits per gram liver per minute) efflux. At the end of the perfusion, liver samples were taken to determine the content of GSH [19] and LDH [21] activity, to express the net sinusoidal rates of GSH or LDH release in relation to the intrahepatic values (fractional rates of efflux, in percent per hour).

Biochemical parameters related to liver cell injury and morphological studies

Animals were anesthetized with 50 mg Nembutal/kg, i.p., and serum GOT and GPT activities were determined in heparinized serum obtained from blood sampled by cardiac puncture, using the GRANUTEST® 25 12162 and 12185, respectively (E. Merck, Darmstdt, Germany).

The assessment of PMN infiltration in the liver was performed in separate groups of rats, previously perfused *in situ* with 200 mL of a cold solution containing 150 mM KCl and 5 mM Tris buffer, pH 7.4, to remove blood. For this purpose, MPO activity was determined in liver homogenates (20%, w/v) prepared in the above buffer, centrifuged at 20,000 *g* for 15 min at 5°, the pellet being resuspended and homogenized in an equal volume of 50 mM potassium phosphate buffer, pH 5.4, containing 0.5% hexadecyltrimethylammonium bromide [22]. After centrifugation at 20,000 *g* for 15 min at 5°, the supernatant was diluted 1:1 or 1:2 in 182 mM sodium phosphate buffer, pH 5.4. Aliquots of 0.01 or 0.02 mL of this diluted supernatant were added to a reaction medium containing 1.6 mM tetramethylbenzidine, 3 mM hydrogen peroxide, 57 mM KCl, 1.9 mM Tris in 182 mM sodium phosphate buffer, pH 5.4, in a final volume of 1 mL [23]. The reaction was followed at 655 nm and 37°, and the results were expressed as ΔA per minute either per milligram protein, gram of liver, or 100 g body weight. In addition to MPO measurements, hepatic phagocytic activity was assayed by the opsonized zymosan-induced luminol-amplified chemiluminescence [24] of liver homogenates. For this purpose, liver homogenates (25%, w/v) were prepared in complete veronal buffer, pH 7.25 (130 mM NaCl, 4.3 mM KCl, 5 mM sodium 5,5-diethyl barbiturate, 0.5 mM $MgCl_2$ 0.45 mM $CaCl_2$, 1 mg/mL albumin, and 5.6 mM D-glucose, sterilized by filtration). Plasma obtained by centrifugation of blood samples at 2500 *g* for 10 min at 4° was used to opsonize zymosan as described by Allen *et al.* [24], which was resuspended in veronal buffer to give a final concentration of 2.5 mg/mL. Visible chemiluminescence was measured in a Beckman LS 6000TA liquid scintillation spectrometer, using single photon monitoring (Beckman Instruments Corp., Fullerton, CA), at 25°. Measurements were carried out in a reaction medium (final volume of 2 mL) containing veronal buffer, 0.05 mL of 0.8 mM luminol, and 0.02 mL of liver homogenate (1 mg protein). The reaction was started by the addition of 0.05 mL of 2.5 mg/mL opsonized zymosan (time zero), and light emission was recorded at 1-min intervals until peak values were obtained (15 min). Backgrounds obtained under similar conditions in the absence of opsonized zymosan were subtracted from the values found in the complete reaction medium. Cor-

rected light emission (cpm) between 1 and 15 min was integrated and expressed as counts per either milligram of protein, gram of liver, or 100 g body weight.

Light microscopy studies were carried out in liver samples from groups (N = 3–4) of control rats and animals given T_3, lindane, or T_3 plus lindane, as described above. Livers were fixed in 10% formaldehyde, paraffin embedded, and sections were stained with hematoxylin-eosin.

Chemicals and reagents used were obtained from either the Sigma Chemical Co. (St. Louis, MO, U.S.A.), E. Merck (Germany) (GOT and GPT kits), or BDH Ltd. (England) (lindane).

Statistical analysis

Values shown are means ± SEM for the number of separate experiments indicated. The statistical significance of differences among multiple groups was made by one-way ANOVA and the Newman–Keuls' test.

RESULTS

T_3 administration to fed rats for 3 consecutive days elicited a significant increment in its serum levels over control values; lindane treatment did not alter serum T_3 in control rats, but it significantly enhanced (87%) the serum levels of the hormone in T_3-treated animals (Table 1). Concomitantly, T_3 administration increased the metabolic rate of the animals, as shown by the significant increment in their rectal temperature, independently of lindane intoxication (Table 1). This calorigenic effect of T_3 was accompanied by parallel increases in the rate of O_2 consumption in the liver compared with control values, a parameter that was not modified further by lindane treatment (Table 1). Under these conditions, hyperthyroid rats exhibited significantly lower levels of lindane in serum, liver, and adipose tissue, than the respective control group (Table 1).

The study of the effects of T_3 treatment on hepatic parameters related to oxidative stress revealed a 52%

Table 1. Serum T_3 levels, serum and tissue levels of lindane, and parameters related to thyroid calorigenesis, liver oxidative stress status, and hepatocellular injury in control rats and animals subjected to T_3 and/or lindane administration

Parameters	Experimental groups*			
	CC (a)	CL (b)	T_3C (c)	T_3L (d)
Serum T_3 (ng/dL) (N = 5)	82 ± 13†	74 ± 18 (c,d)	311 ± 28 (a,b,d)	582 ± 97 (a,b,c)
Rectal temperature (°) (N = 22)	37.6 ± 0.09	37.9 ± 0.09 (c,d)	38.6 ± 0.1 (a,b)	38.7 ± 0.07 (a,b)
Liver O_2 uptake (μmol/g liver/min) (N = 6)	1.82 ± 0.08	1.85 ± 0.06 (c,d)	2.76 ± 0.17 (a,b)	2.75 ± 0.1 (a,b)
Serum lindane (μg/mL) (N = 6)	ND‡	0.74 ± 0.06 (d)	ND	0.41 ± 0.07 (b)
Liver lindane content (μg/g) (N = 6)	ND	2.68 ± 0.27 (d)	ND	1.97 ± 0.09 (b)
Adipose tissue lindane content (μg/g) (N = 6)	ND	196 ± 11 (d)	ND	155 ± 7 (b)
Liver GSH content (μmol/g liver) (N = 6)	8.17 ± 0.36	7.09 ± 0.70 (c,d)	3.90 ± 0.56 (a,b,d)	1.97 ± 0.25 (a,b,c)
Liver TBARS formation (nmol/mg protein/hr) (N = 6)	0.56 ± 0.03	1.04 ± 0.06 (a,d)	1.06 ± 0.19 (a,d)	1.58 ± 0.08 (a,b,c)
Liver TBARS/GSH ratio (N = 6)	0.07 ± 0.006	0.15 ± 0.01 (d)	0.27 ± 0.16 (a,d)	0.80 ± 0.09 (a,b,c)
Fractional liver LDH efflux§ (%/hr) (N = 6)	0.23 ± 0.04	0.17 ± 0.05 (c,d)	3.29 ± 0.63 (a,b,d)	23.2 ± 6.3 (a,b,c)
Fractional liver GSH efflux§ (%/hr) (N = 6)	9.3 ± 1.1	11.1 ± 1.9 (c,d)	29.5 ± 3.1 (a,b,d)	67.8 ± 8.0 (a,b,c)
Serum GOT (U/L) (N = 6)	42 ± 3	41 ± 2 (c,d)	79 ± 8 (a,b,d)	173 ± 19 (a,b,c)
Serum GPT (U/L) (N = 6)	25 ± 2	26 ± 5 (d)	24 ± 1 (d)	40 ± 3 (a,b,c)
Serum GOT/GPT ratio (N = 6)	1.68 ± 0.04	1.58 ± 0.24 (c,d)	3.29 ± 0.31 (a,b,d)	4.33 ± 0.45 (a,b,c)

* CC, control rats (a); CL, lindane-treated rats (b); T_3C, hyperthyroid rats (c); T_3L, hyperthyroid rats treated with lindane (d). See Materials and Methods.

† Results are the means ± SEM for the number (N) of animals indicated. The significance of the differences between mean values ($P < 0.05$) is shown by the letters in parentheses under the values for each experimental group.

‡ ND, not detectable.

§ Fractional LDH efflux was calculated by dividing the net rates of sinusoidal LDH release in liver perfusion studies [CC, 17.6 ± 3.3 mU/g liver/min (N = 6); CL, 10.8 ± 3.4 (N = 6) (c,d); T_3C, 181.6 ± 43.8 (N = 6) (a,b,d); T_3L, 1391.3 ± 384.2 (N = 6) (a,b,c)] by the respective LDH activities in the liver [CC, 463 ± 37 U/g liver (N = 6); CL, 426 ± 30 (N = 6); T_3C, 331 ± 33 (N = 6) (a); T_3L, 359 ± 34 (N = 6)]. Fractional GSH efflux was calculated by dividing the net rates of sinusoidal GSH efflux [CC, 11.9 ± 1.1 nmol/g liver/min (N = 6); CL, 11.8 ± 1.1 (N = 6) (c,d); T_3C, 19.6 ± 1.1 (N = 6) (a,b,d); T_3L, 27.8 ± 3.5 (N = 6) (a,b,c)] by the respective liver GSH levels assessed after perfusion studies [CC, 7.67 ± 0.38 μmol/g liver (N = 6); CL, 6.36 ± 0.57 (N = 6) (c,d); T_3C, 3.98 ± 0.20 (N = 6) (a,b,d); T_3L, 2.46 ± 0.09 (N = 6) (a,b,c)].

reduction in the content of GSH compared with control values, which was depressed further to 24% of the control value by the concomitant treatment with lindane (Table 1); liver GSH was not altered significantly by lindane in animals not subjected to hormone administration (Table 1). In addition, separate T_3 and lindane treatments enhanced liver TBARS formation by 89 and 86% over control values, respectively, while the combined administration elicited a 182% increment (Table 1). From these data, it can be estimated that lindane intoxication in hyperthyroid rats caused a significant 10.6-fold increase in the TBARS formation/GSH content ratio of the liver, compared with that in control animals (Table 1). Comparatively, separate lindane (113%; not significant) or T_3 (294%; $P < 0.05$) treatments produced lower increments in the hepatic TBARS/GSH ratio than that observed in control rats (Table 1). In these conditions, TBARS formation by the liver, among the different experimental groups, exhibited a significant inversed correlation with the logarithmic content of hepatic GSH (Fig. 1).

The influence of lindane and T_3 treatments on the liver sinusoidal rates of LDH and GSH efflux was studied in the isolated perfused rat liver. In these experiments, control animals exhibited a fractional LDH efflux of 0.23 ± 0.04%/hr (N = 6) which was not modified by lindane intoxication, and significantly increased by 13.3- and 100-fold in the livers of rats given T_3 alone or T_3 plus lindane, respectively (Table 1). Similarly, the fractional efflux of GSH in the liver of control rats [9.3 ± 1.1%/hr (N = 6)] was not altered by acute lindane treatment, whereas it was enhanced significantly by 2.2- and 6.3-fold by T_3 alone or T_3 plus lindane, respectively (Table 1). Rates of liver LDH and GSH efflux correlated significantly ($r = 0.98$; $P < 0.0001$). Furthermore, both parameters exhibited significant correlations with the respective liver TBARS/GSH ratios, calculated for the different experimental groups studied (Fig. 2).

The administration of T_3 to fed rats led to a significant (89%) increase in the activity of serum GOT over control values (Table 1). Acute lindane treatment did not modify serum GOT levels when given alone, but it enhanced this enzymatic activity by 3.1-fold in hyperthyroid animals (Table 1). Serum GPT activity was not altered in rats subjected to separate lindane or T_3 treatments; however, their combined administration produced a 45% increment compared with control animals (Table 1). Accordingly, the serum GOT/GPT ratio was not changed significantly by lindane (net increase of 0.06), whereas it was enhanced by T_3 administration (net increase of 1.77; $P < 0.05$) and by the joint T_3 and lindane treatment (net increase of 2.82; $P < 0.05$) over control values (Table 1).

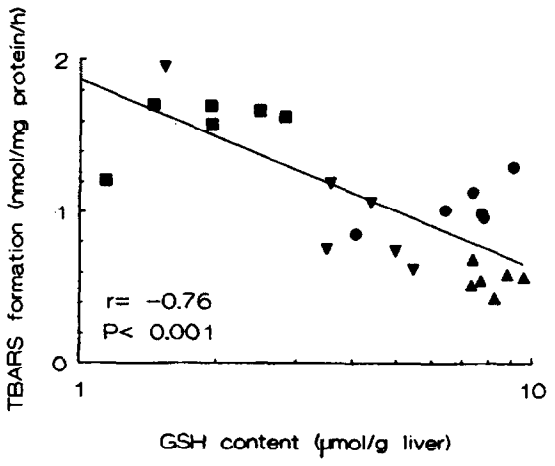

Fig. 1. Correlation between the rate of thiobarbituric acid reactants (TBARS) formation and the respective glutathione content (GSH) in the liver of control rats and animals subjected to T_3 and/or lindane administration. Animals were divided into four groups: controls (▲), lindane-treated rats (●), hyperthyroid rats (▼), and hyperthyroid rats treated with lindane (■), as described in Materials and Methods. Regression line: Y = 1.83 − 1.20 log X.

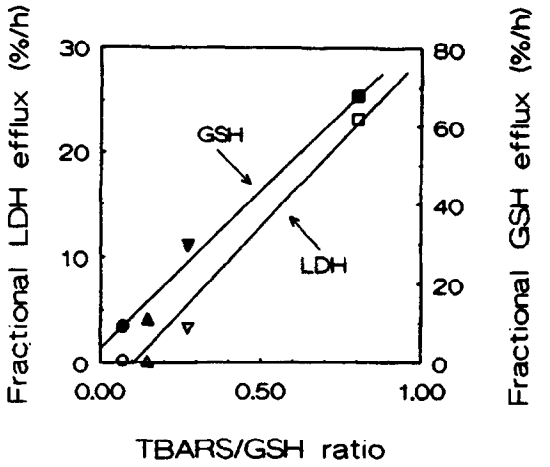

Fig. 2. Correlations between the fractional rates of lactate dehydrogenase (LDH) or glutathione (GSH) efflux from isolated perfused livers and the thiobarbituric acid reactants (TBARS) formation/hepatic GSH content ratios in control rats and animals subjected to T_3 and/or lindane administration. Animals were divided into four groups: control rats (○, ●), lindane-treated rats (△, ▲), hyperthyroid rats (▽, ▼), and hyperthyroid rats treated with lindane (□, ■). Regression lines from average data presented in Table 1: LDH efflux, Y = −4 + 33.2X (r = 0.98; $P < 0.0001$); GSH efflux, Y = 3.1 + 81.6X ($r = 0.99$; $P <$ 0.001).

The light microscopy study of the liver from rats subjected to the different treatments is shown in Fig. 3. As can be seen, livers from control animals (Fig. 3A) and rats given a single dose of lindane (Fig. 3B) were normal. Kupffer cell hyperplasia was present in the liver of hyperthyroid animals, in the form of focal aggregates within the lobule (Fig. 3C). More severe hepatic lesions were observed in hyperthyroid rats treated with lindane, including extensive zones of coagulative-type necrosis of liver cells (Fig. 3D), and necrosis of isolated hepatocytes with the presence of circumscribed collections of inflammatory cells, predominantly Kupffer cells, lymphocytes, and polymorphonuclear leukocytes (Fig. 4). These granulomas were observed anywhere within the hepatic lobule, without relation to vascular structures (Fig. 4).

Table 2 shows the influence of T_3 and/or lindane administration on rat liver MPO activity. As can be seen, hyperthyroid animals and rats acutely treated with lindane exhibited liver MPO activities comparable with control values. However, the combined T_3 and lindane treatment elicited significant increases in hepatic MPO

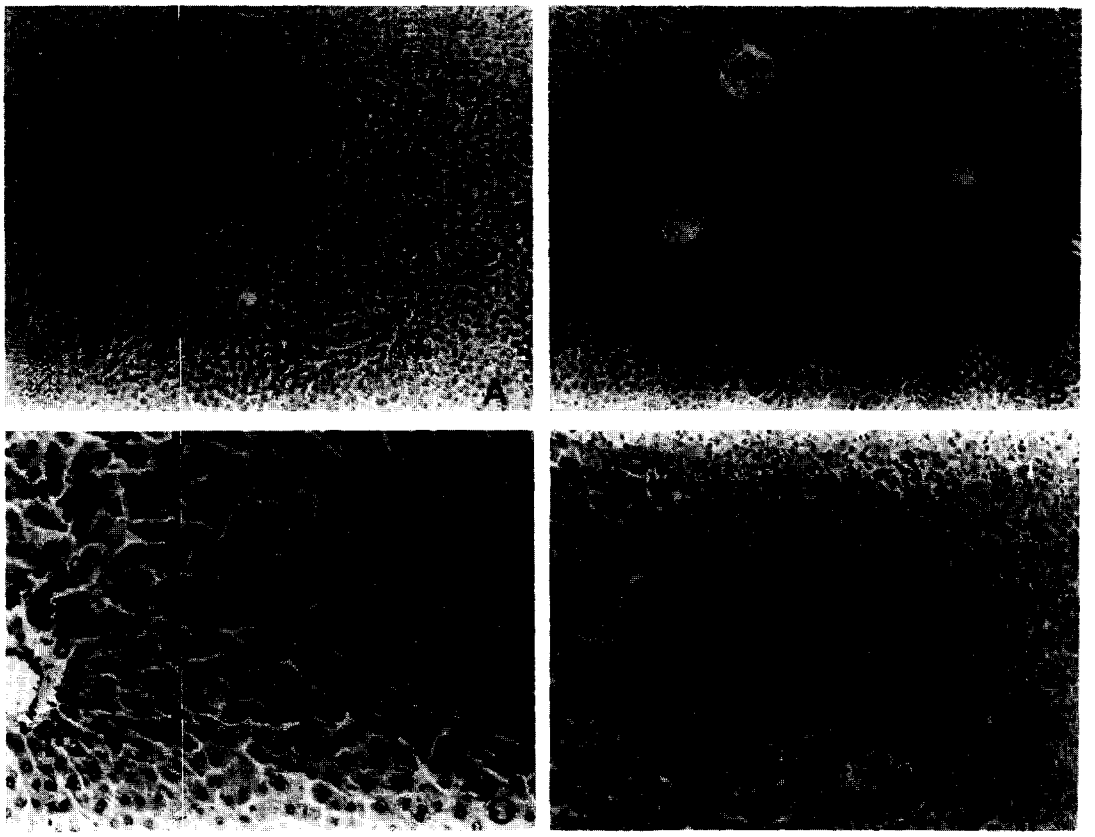

Fig. 3. Structural characteristics of liver parenchyma in a control rat (CC; hematoxylin-eosin, magnification ×30) (A) and in animals subjected to either lindane (CL; hematoxylin-eosin, magnification ×30) (B), T_3 (T_3C; hematoxylin-eosin, magnification ×80) (C), or T_3 and lindane (T_3L; hematoxylin-eosin, magnification ×80) (D) administration, as described in Materials and Methods.

activity over controls, either when expressed as ΔA/min per mg of protein (157%), per g of liver (67%), or per 100 g body weight (88%) (Table 2).

Opsonized zymosan induced a chemiluminescent response in rat liver homogenates, with a maximal light emission produced at about 15 min after the addition of the chemiluminigenic probe (Fig. 5). Corrected data shown in Fig. 5 for control rats were obtained by subtracting the luminescence emitted by liver homogenates in the absence of zymosan, which slowly increased in time (not shown), comprising 10% of that found in its presence [integrated chemiluminescence between 1 and 15 min in the absence of zymosan, 7 ± 1 counts/mg protein (N = 5); integrated light emission in the presence of zymosan, 73 ± 8 (N = 5)]. Under these conditions, hyperthyroidism achieved a significant enhancement in total chemiluminescence of liver homogenates over control values (Fig. 5; Table 3), independently of lindane treatment (Table 3), either when expressed as counts per mg of protein, per g of liver, or per 100 g body weight (Table 3). Lindane administration resulted in hepatic opsonized zymosan-dependent light emission not significantly different from that observed in controls (Table 3).

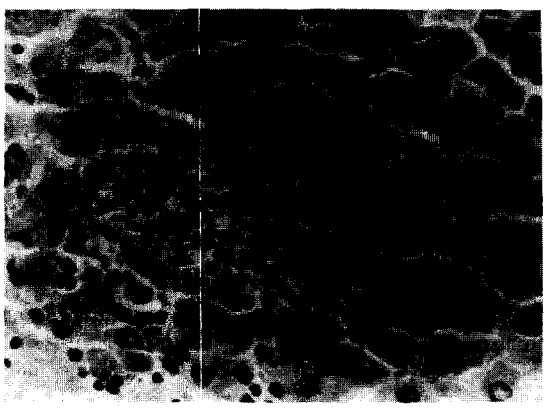

Fig. 4. Histopathology of rat liver after the joint administration of T_3 and lindane (T_3L). The section shows a granuloma and isolated necrotic hepatocytes (hematoxylin-eosin, magnification ×200).

DISCUSSION

T_3 and lindane interactions after their in vivo administration

The joint treatment with T_3 and lindane elicited marked changes in their circulating levels, compared with those achieved by the separate administration. Higher serum T_3 levels were observed in rats given T_3 and lindane than those found in animals treated with T_3 alone. This effect of lindane could be due to an inhibition of the uptake and/or catabolism of T_3 by the liver, the main organ responsible for thyroid hormone metabolism [25], thus determining higher levels of T_3 in serum.

Table 2. Myeloperoxidase (MPO) activity of livers from control rats and animals subjected to T_3 and/or lindane administration

	Experimental groups*			
MPO activity	CC (a)	CL (b)	T_3C (c)	T_3L (d)
ΔA/min/mg protein	0.42 ± 0.04†	0.43 ± 0.05 (d)	0.54 ± 0.05 (d)	1.08 ± 0.20 (a,b,c)
ΔA/min/g liver	67.7 ± 10.4	69.0 ± 5.1 (d)	65.5 ± 3.6 (d)	113.1 ± 15.3 (a,b,c)
ΔA/min/100 g body weight	267 ± 56	260 ± 17 (d)	243 ± 16 (d)	502 ± 82 (a,b,c)

* CC, control rats (a); CL, lindane-treated rats (b); T_3C, hyperthyroid rats (c); T_3L, hyperthyroid rats treated with lindane (d). See Materials and Methods.

† Results are the means ± SEM for five animals per group. The significance of the differences between mean values ($P < 0.05$) is shown by the letters in parentheses under the values for experimental group.

These processes are mediated by membrane-bound systems, present either in the plasma membrane of the hepatocyte (energy-dependent transport system for iodothyronines) [26] or in the endoplasmic reticulum (iodothyronine deiodinases) [25]. In agreement with this suggestion, lindane is known to perturb the activity of several unrelated membrane-bound enzymes, due to its insertion into the lipid domains surrounding the integral proteins of membranes [27]. However, the findings that combined T_3 and lindane treatment elicited a calorigenic response with increased rates of hepatic O_2 uptake over controls, at values comparable to those observed in rats given T_3 alone, suggest that liver T_3 uptake is not the main process affected by lindane. Also, the effect is unlikely to be due to stimulation of the endogenous production of T_3 by lindane, as animals treated with the insecticide have serum T_3 levels comparable to those in control rats. Thus, future studies on the influence of the insecticide on T_3 catabolism in the liver and other target tissues [25] are needed to establish the relevance of this process, as a major factor enhancing serum T_3 levels.

Contrary to the increasing effect of lindane on serum T_3 levels upon their combined treatment, hyperthyroidism resulted in a significant diminution in the serum levels of lindane, over values found in rats given lindane alone. The development of a hyperthyroid state comprising an enhanced oxidative activity in the liver results in the proliferation of the smooth endoplasmic reticulum,

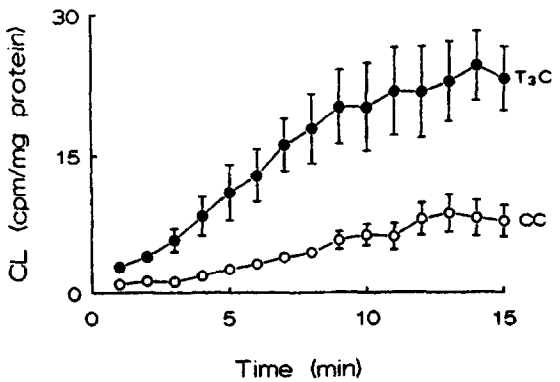

Fig. 5. Opsonized zymosan-induced luminol-amplified chemiluminescence (CL) of liver homogenates from control rats (CC) and hyperthyroid animals (T_3C). Results are the means ± SEM for five rats per group.

with induction of microsomal functions related to NADPH-dependent electron transport reactions [11]. Thus, lower levels of lindane in serum and liver by T_3 treatment may reflect an accelerated microsomal biotransformation of the insecticide in the liver, leading to a diminished availability of lindane for storage in the adipose tissue (Table 1). This view is in line with the enhanced biotransformation of xenobiotics reported in hyperthyroid animals [11] and humans [28]. Hyperthyroidism also increases cardiac output, hepatic and renal blood flow, and the glomerular filtration rate [29], factors that may affect drug distribution and elimination, in addition to changes in biotransformation. Direct interactions of T_3 with the microsomal enzymes involved in lindane biotransformation seems unlikely, as both compounds are catabolized in the liver by different specific pathways present in this subcellular fraction [5, 11].

T_3 and lindane interaction, liver oxidative stress status, and hepatocellular injury

Parameters related to oxidative stress were studied in the liver of control and hyperthyroid rats, subjected to a single dose of 20 mg lindane/kg. This dose of lindane has been demonstrated previously to produce a 20% increase in the content of liver microsomal cytochrome P450, without significant changes in microsomal $O_2^{\cdot-}$ generation or in the activity of hepatic enzymes affording antioxidant protection, compared with higher doses (40–80 mg/kg) [9]. Under these conditions, the hepatic content of GSH is not altered by lindane, while lipid peroxidation is increased, a finding that may be associated with a greater production of lindane-derived reactive metabolites upon cytochrome P450 induction [6]. The relationship between the lipid peroxidation rate, as a free radical-mediated process, and the content of the major antioxidant cellular component GSH, is indicative of the oxidative stress status of the hepatocyte. Thus, low doses of lindane do not alter the oxidative stress status of the liver, as evidenced by the lack of changes in the TBARS/GSH ratio compared with control values. This observation correlates both with the constancy of serum transaminases and of the fractional rates of liver sinusoidal LDH and GSH efflux, and with the lack of morphological evidence of hepatic cell injury.

As previously demonstrated [12, 30, 31], T_3 treatment led to a significant increase in the rate of O_2 consumption and in the oxidative stress status of the rat liver. Under these conditions, hyperthyroid animals exhibit

Table 3. Opsonized zymosan-induced luminol-amplified chemiluminescence of liver homogenates from control rats and animals subjected to T_3 and/or lindane administration

Integrated chemiluminescence*	Experimental groups†			
	CC (a)	CL (b)	T_3C (c)	T_3L (d)
Counts/mg protein (10^{-3})	66 ± 8‡	62 ± 15 (c,d)	221 ± 32 (a,b)	189 ± 24 (a,b)
Counts/g liver (10^{-6})	14 ± 2	12 ± 3 (c,d)	57 ± 10 (a,b)	40 ± 7 (a,b)
Counts/100 g body weight (10^{-6})	53 ± 8	53 ± 18 (c,d)	189 ± 41 (a,b)	152 ± 28 (a,b)

* Corresponds to the area under the time curves of light emission between 1 and 15 min, corrected for backgrounds obtained in the absence of zymosan.

† CC, control rats (a); CL, lindane-treated rats (b); T_3C, hyperthyroid rats (c); T_3L, hyperthyroid rats treated with lindane (d). See Materials and Methods.

‡ Results are the means ± SEM for five animals per group. The significance of the differences between mean values ($P < 0.05$) is shown by the letters in parentheses under the values for each experimental group.

higher serum GOT activities compared with control values, in addition to the elevated sinusoidal efflux of LDH (Table 1) [30], GSH (Table 1) [30], and protein [30] observed in liver perfusion studies. These observations indicate that thyroid calorigenesis leads to a permeabilization of the plasma membrane of the hepatocyte, which may result from the prooxidant condition induced. In agreement with this view, increased serum levels of liver enzymes, namely alkaline phosphatase [32–34] and γ-glutamyl transpeptidase [35], have been described in patients with hyperthyroidism. The latter changes are observed in addition to abnormalities in liver function tests (elevated serum bilirubin levels [32, 36] and bromosulphthalein retention [37] and decreased prothrombin time [32]) and in circulating and urinary lipid peroxidation indicators [38, 39]. Thus, thyroid hormone-induced liver oxidative stress may represent a damaging mechanism involving a free radical attack of membrane phospholipids, that would place the organ in an abnormal functional state, more susceptible to additional toxic aggressions. A major histological finding in this work is the appearance of Kupffer cell hyperplasia in the livers of rats subjected to T_3 treatment (Fig. 3C). These macrophages residing in the sinusoids of the liver produce reactive O_2 species, in addition to cytotoxic polypeptides such as interferon α/β, interleukin I, and tumor necrosis factor by suitable activators [18]. In fact, stimulated Kupffer cells have been shown to generate $O_2^{\cdot -}$ and nitric oxide, without production of hypochlorous acid due to lack of MPO activity [40]. Kupffer cell hyperplasia induced by hyperthyroidism correlates with the enhanced opsonized zymosan-induced luminol-amplified chemiluminescence of liver homogenates over control values. This experimental approach assesses the generation of short-lived free radicals and excited states associated with phagocyte respiratory burst activity [24, 40]. In these studies, the observed luminescent response does not seem to be related to infiltration of PMN in the liver, as shown histologically and by the lack of changes in hepatic MPO activity in T_3-treated rats over control values. Thus, hyperthyroidism in the rat leads to an enhancement in the number of Kupffer cells which, upon stimulation, would represent an alternative source of reactive O_2 species to that induced at different subcellular sites in parenchymal cells [11–13]. The prooxidant condition elicited in hepatocytes by hyperthyroidism probably reflects an enhancement in aerobic metabolism, triggered by the interaction of thyroid hormones with nuclear receptors leading to an increased synthesis of related enzymatic systems [41]. In this respect, studies by Sellitti *et al.* [42] on the antibody characterization and immunofluorescent localization of nuclear thyroid hormone-binding proteins revealed an intense fluorescence in the cells lining the hepatic sinusoids. This observation raises the possibility that hyperthyroidism may increase the respiratory burst activity of Kupffer cells by induction of the enzymatic mechanisms involved, as shown for PMN [43], in addition to the enhancement in macrophage number. However, further studies are needed to clarify this view.

Acute lindane administration to hyperthyroid animals further enhanced the oxidative stress status of the liver over that induced in rats given T_3 alone. The observed net increases in the TBARS/GSH ratio by lindane (0.08), T_3 (0.20), and T_3 plus lindane (0.73) (Table 1) indicate that hyperthyroidism potentiates the oxidative stress status in the liver of lindane-treated rats, compared with that elicited by the separate treatments. This view is in line with the significant inversed correlation between hepatic TBARS formation and the logarithm of GSH content, established for the different experimental groups studied (Fig. 1). Furthermore, potentiation of liver oxidative stress in the livers of lindane-treated rats by hyperthyroidism is produced concomitantly with an exacerbation in the derangement of the permeability properties of the plasma membrane of the hepatocyte. This is evidenced by the drastic elevation in the sinusoidal efflux of LDH and GSH produced by the combined treatment, exceeding the sum of the effects elicited by the separate treatments, changes that significantly correlate with those observed in the TBARS/GSH ratio of the liver. In these conditions, increments in the sinusoidal efflux of GSH are unlikely to occur through the concentration-dependent carrier-mediated process, characterized by a sigmoidal-type kinetics [44], due to the significant diminution in the intrahepatic GSH levels produced (Table 1). In agreement with the above findings, hyperthyroid animals given lindane exhibit a significant elevation in serum GOT and GPT activities, with higher GOT/GPT ratios, compared with the respective control values, coupled to the incidence of extensive hepatocellular necrosis. In addition, rats subjected to the combined

T_3 and lindane treatment exhibit an inflammatory type of granulomatous liver injury, with aggregates containing lymphocytes, PMN, and Kupffer cells (Fig. 4). In this situation, infiltration of PMN agrees with the enhancement in hepatic MPO activity. Furthermore, the increased number of PMN and/or Kupffer cells in these granulomas may explain the elevated hepatic luminol-amplified chemiluminescence induced by opsonized zymosan. Thus, Kupffer cell hyperplasia and PMN infiltration may represent an alternative source of free radical activity in the liver of T_3 and lindane-treated animals, besides those previously identified in liver cells after T_3 treatment [11–13].

In conclusion, data presented indicate that the joint administration of T_3 and lindane to rats alters the availability of both agents, compared with the separate treatments. Increased serum T_3 by lindane is likely to sustain the calorigenic action of the hormone on the liver, known to involve an enhanced free radical activity [12, 13]. In addition, decreased serum and hepatic lindane levels by T_3 would imply an accelerated biotransformation of the insecticide, known to involve the generation of reactive metabolites [5]. These actions may explain the increased susceptibility of the liver to the toxic effects of lindane in hyperthyroid animals. This seems to be accomplished through potentiation of the oxidative stress status of the liver, leading to marked increases in serum transaminases assessed *in vivo* and in the sinusoidal efflux of LDH and GSH measured in liver perfusion studies. In these conditions, liver oxidative stress seems to be related mainly to an enhanced T_3-induced free radical generation at hepatic microsomal, mitochondrial, and peroxisomal sites [11–13], but it could also be mediated secondarily by an increased phagocytic activity, coupled to Kupffer cell hyperplasia and PMN infiltration. In line with this view, hyperthyroidism in experimental animals has been reported to increase the sensitivity of the liver to anoxia [45], chloroform [46], and carbon tetrachloride [47], and potentiates the toxicity of ozone and nitrogen dioxide in the lung [48], conditions that involve free radical interactions among other mechanisms of cell injury. The molecular mechanisms underlying the potentiating effect of hyperthyroidism on lindane hepatotoxicity are currently under study in our laboratories.

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