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Short Communication

Changes in lipoprotein lipase activity and plasma liver lipids in thiram intoxicated rats*

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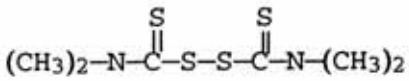
Acute thiram (tetramethyl-bis-thiocarbamyl disulphide) poisoning of rat (a single dose of 50% LD_{50}) caused decreased lipoprotein lipase (LPL) activity in adipose tissue, the greatest inhibition being observed at 72 h after administration of the pesticide. Simultaneously, the levels of total plasma cholesterol, triacylglycerols and the high density lipoprotein (HDL) cholesterol were increased.

On repeated pesticide administration (5% LD_{50}) decreased LPL activity was observed after 14 and 30 days of poisoning, whereas after 90 days the LPL activity was distinctly increased. The levels of total cholesterol (in all periods of poisoning) and HDL cholesterol (only after 30 days of poisoning) became increased. These changes were accompanied by decreased content of free fatty acids and increase of hepatic triacylglycerols. The changes observed in the lipoprotein lipase activity of thiram-poisoned rats correspond to the profiles of plasma lipoproteins typical of thyroid hypofunction.

Thiram (tetramethyl-bis-thiocarbamyl disulphide),



belongs to the dithiocarbamate class of fungicides. This seems to be of special importance due to the ability of thiram to interact with sulfhydryl-containing compounds, which led to its wide practical application in medicine and agriculture.

Some recent studies indicate that it has both a neurotoxic [1, 2], teratogenic [3] and mutagenic [4 - 6] effect. Thiram is also known to affect carbohydrate [7 - 9] and protein metabolism [10 - 14]. However, so far very few studies concerned the effect of this fungicide on lipid metabolism [15 - 17].

This paper describes the effect of thiram on the activity of lipoprotein lipase from adipose tissue, and the level of lipids in blood plasma and liver of the rat.

MATERIALS AND METHODS

White male Wistar rats weighing 160 - 200 g were used for experiments. The animals were fed a standard LSM mixture (Bacutil, Warsaw) and water *ad libitum*. In acute intoxication the rats were given thiram (analytical standard produced by Organica-Azot, Poland) *per os* in a single dose of 50% LD_{50} (290 mg/kg body weight), and were killed by decapitation 12, 24, 48 and 72 h after administration of the pesticide. In repeated administration of small doses (5% LD_{50}) the animals were given thiram six times weekly for 14, 28 and 90 days. The control animals received corresponding amounts of corn oil.

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[1]Abbreviations: FFA, free fatty acids; HDL, high density lipoprotein; LPL, lipoprotein lipase.

Plasma free fatty acids (FFA[1]) were measured according to Duncombe [18]. Lipids were extracted as described by Carlson [19], and triacylglycerols [20] and total cholesterol [21] were determined. High density lipoprotein (HDL) cholesterol was measured after precipitation of other lipoprotein fractions with phosphotungstate in the presence of Mg^{2+} [22].

Lipoprotein lipase activity was measured in epididymal adipose tissue of fed animals [23] using Ediol (Calbiochem) as a substrate. The activity was expressed in micromoles of FFA released per 1 h by 1 g of tissue [18].

All results were analysed statistically by means of Student's *t*-test.

RESULTS

Acute poisoning of rats with thiram (a single dose of 50% LD_{50}) led after 48 h to a significant increase in the content of triacylglycerols and total cholesterol in their blood plasma (Fig. 1). The increased total cholesterol content persisted even 72 h after administration of the pesticide. Moreover, an increase in HDL cholesterol was observed beginning with 24 h after poisoning, and the content of free fatty acids was lowered after all the time intervals studied.

In the animals intoxicated with repeated small doses of thiram (5% LD_{50}) the content of total cholesterol in plasma was increased throughout the time of the experiment, and a statistically significant increase in HDL cholesterol was observed after 30 days of poisoning (Fig. 2). On the other hand, the plasma content of free fatty acids was significantly decreased after 14, 30, as well as 90 days of poisoning.

A marked increase in the content of triacylglycerols in liver was observed both in the case of the single dose (Fig. 3 A) and repeated intoxication with thiram (Fig. 3 B). On the other hand, the differences in the content of total cholesterol between control and the thiram-poisoned rats were observed only after 24 and 48 h (Fig. 3 A).

The lipoprotein lipase activity in adipose tissue was significantly lowered both in acute (Table 1) and repeated intoxication with thiram (Table 2). Only after 90 days of administration of the 5% LD_{50} dose, an increase in the activity was observed.

DISCUSSION

Morphological and biochemical studies have shown that thiram intoxication results in injuries of the liver structure and impairment of

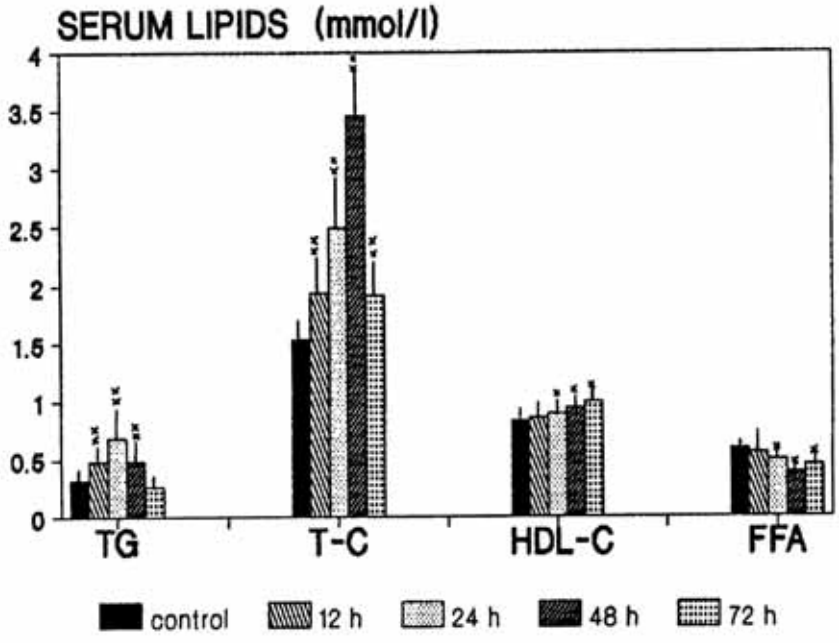

Fig. 1. Changes in the content of triacylglycerols (TG), total cholesterol (T-C), HDL cholesterol (HDL-C) and free fatty acids (FFA) in rat serum after acute thiram poisoning.
Mean values ± S.E., n = 10. Significance of differences from control groups: $^{*}P \leq 0.05$; $^{**}P \leq 0.01$.

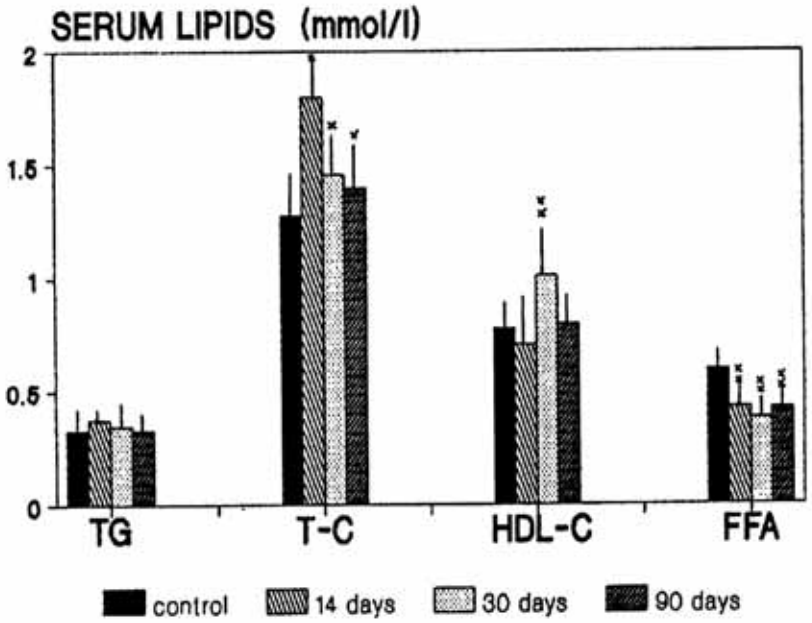

Fig. 2. Changes in the content of triacylglycerols (TG), total cholesterol (T-C), HDL cholesterol (HDL-C) and free fatty acids (FFA) in rat serum after chronic thiram poisoning.
Mean values ± S.E., n = 10 (control, n = 30). Significance of differences from control groups: $^{*}P \leq 0.05$; $^{**}P \leq 0.01$.

the hepatic function. This is expressed by increased concentration of glycogen, triacylglycerols and long-chain fatty acyl-CoA in rat liver [16]. In the present work a high increase in the content of triacylglycerols in rat liver was observed both after administration of a single high dose of thiram and after prolonged administration of lower doses. The results obtained are in agreement with the observations of Faudemay *et al.* [16] who suggested that thiram inhibits glycolysis at the step of glyceraldehyde-3-phosphate oxidation, this inhibition leading, in turn, to increased synthesis of glycerol 3-phosphate.

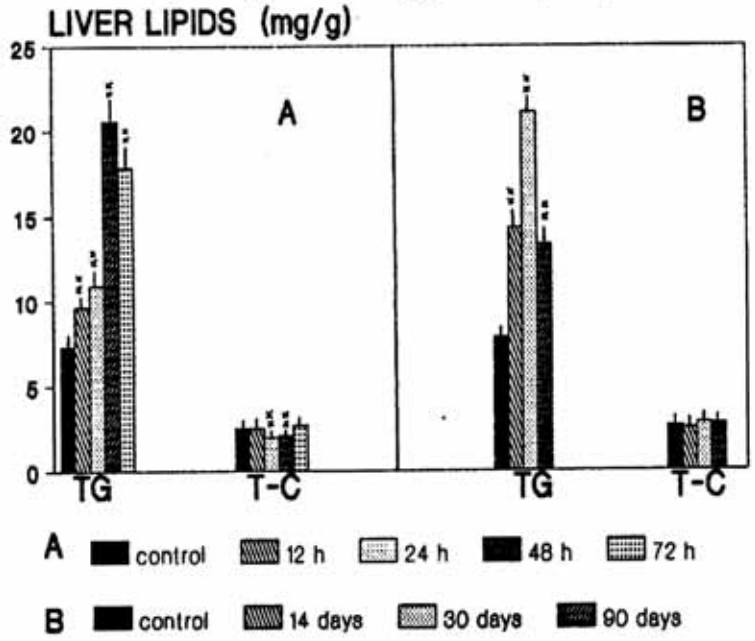

Fig. 3. Changes in rat hepatic triacylglycerol (TG), total cholesterol (T-C) content after thiram poisoning.
A, Acute poisoning; B, chronic poisoning. Mean values ± S.E., n = 10 (control, n = 30). Significance of differences from control groups: $^{**}P \leq 0.01$.

Table 1
Effect of a single dose of thiram (50% LD_{50}) on the adipose tissue lipoprotein lipase (LPL) activity in the rat

Time after administration (h)	LPL activity (μmol FFA × h^{-1} per g tissue)
0	12.1 ± 1.02
12	7.0 ± 0.50*
24	5.8 ± 0.45*
48	4.4 ± 0.60*
72	2.6 ± 0.26*

Mean values of LPL activity ± S.E. in ten animals are presented.
* Significantly different from the control, $P \leq 0.05$.

Lowering of the content of free fatty acids in circulation, demonstrated in the present work, could be due to their increased uptake by the liver and increased esterification to triacylglycerols. The lipid reserves of the adipose tissue become liberated only as a subsequent step, as proved by Faudemay *et al.* [16] who, in rats repeatedly intoxicated with thiram, observed decreased weight of the adipose tissue.

The hypercholesterolemia observed by us in thiram intoxicated rats shows a distinct similarity to severe abnormalities in lipoprotein metabolism found in hypothyroidism [24]. Dithiocarbamates including thiram are known to have goitrogenic properties [25]; their effect is due to biotransformation to alkyl derivatives of thiourea, a compound known to have antithyroid activity.

One of the factors contributing to the dyslipoproteinemia observed in hypothyroidism, is the altered activity of lipoprotein lipase. However, a comparison of the data for man and rat makes evident distinct differences between them with respect to thyroid function and lipoprotein lipase activity. Hypothyroidism in man is accompanied by decreased lipoprotein lipase activity in adipose tissue [26]. In contrast, experimental hypothyroidism in the rat leads to an increase of the enzyme activity in that tissue [27, 28].

The lowered LPL activity observed by us in the adipose tissue of rats intoxicated with thiram, both after administration of small doses for 14 or 30 days, points to deficiency of thyroid hormones, analogous to that observed in hypothyroidism in man. However, it should be noted that after 90 days of thiram administration in doses of 5% LD_{50} the LPL activity in rat adipose tissue was increased.

The changes in LPL activity led to impaired degradation of triacylglycerols-rich lipoproteins. Dolphin & Forsyth [24] demonstrated

Table 2
Effect of repeated poisoning with thiram (5% LD_{50}) on the adipose tissue lipoprotein lipase (LPL) activity in the rat

Period of poisoning (days)	Group	LPL activity (μmol FFA × h^{-1} per g tissue)
14	Control	12.7 ± 0.95
	Intoxicated	6.9 ± 0.59*
30	Control	9.6 ± 0.50
	Intoxicated	6.4 ± 1.09*
90	Control	11.1 ± 0.85
	Intoxicated	15.4 ± 0.65*

Mean values of LPL activity ± S.E. in ten animals are presented.
* Significantly different from the control, $P \leq 0.05$.

that the serum very low density lipoproteins (VLDL) of hypothyroid rats are less rich in triacylglycerols and contain relatively more of cholesteryl esters than the normal serum very low density lipoproteins. Also the elimination of cholesterol from circulation to the liver *via* the HDL system is impaired in hypothyroidism, being expressed by the increased total HDL cholesterol concentration. This also was confirmed by our studies. However, it cannot be excluded that thiram has a direct toxic effect on liver cells, resulting in changes in the activity of hepatic lipase and lecithin-cholesterol acyltransferase, the enzymes involved in lipoprotein metabolism.

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