

Effect of Propylthiouracil on Liver Regeneration in Rats after Partial Hepatectomy

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Summary

The effect of propylthiouracil (PTU) on the growth activity of intact liver and liver regenerating after partial (65-70 %) hepatectomy (PH) was studied in rats. PTU (Propycil, Kali-Chemie, FRG) was dissolved in drinking water (1 g PTU per litre) and this was given to the rats, as their sole source of fluids, three days before PH and then up to the end of the experiment. In rats given PTU, marked inhibition of liver DNA synthesis and the mitotic activity of hepatocytes was found after PH. This effect was potentiated to some extent by partial inanition of the experimental animals given PTU, as demonstrated in a paired feeding test in control rats. PTU inhibition of DNA synthesis in intact and regenerating liver also took effect in thyroidectomized rats, even with substitution (thyroid hormone) therapy. The experiments demonstrated that the effect of propylthiouracil on DNA synthesis in the liver is mediated primarily by way of its direct effect on the liver.

Key words

Propylthiouracil – Thyroidectomy – Partial hepatectomy – Liver regeneration – DNA synthesis – Mitotic index

Introduction

Regeneration of the liver after partial hepatectomy is controlled by a complex humoral mechanism. It has been demonstrated experimentally that an important role in this mechanism is played by insulin, glucagon, epidermal growth factor (Leffert *et al.* 1988) and a number of newly discovered substances (hepatopoietins – Michalopoulos 1990) with a markedly specific effect on liver regeneration. It has further been demonstrated that the thyroid hormones also participate in the stimulation of liver regeneration (Červinková *et al.* 1984) and that their administration induces a growth effect in both intact and regenerating liver tissue manifested by stimulation of DNA synthesis and mitotic activity in the hepatocytes (Lee *et al.* 1968, Short *et al.* 1980 a, b). The effect of reduced thyroid hormone synthesis on the regenerative capacity of the liver has not been sufficiently analysed experimentally, however, despite the obvious practical significance of this question.

The available data show inconsistencies in the effect of thiouracil used to influence thyroid function on the initiation of liver regeneration. Canzanelli *et al.* (1949) described inhibition of liver regeneration after administering thiouracil. Drabkin (1950) found that it had no effect on regeneration, while Fogelman and Ivy (1948) described a stimulating effect.

In our studies of these problems, we first of all induced hypothyroidism with propylthiouracil, with reference to its use in the clinical treatment of conditions accompanied by hyperthyroidism. The results of these experiments prompted us, however, also to verify the effect of propylthiouracil on the initiation of liver regeneration in thyroidectomized rats.

Material and Methods

The experiments were carried out on male and female rats with an initial body mass of 180-210 g, which were fed *ad libitum* on a standard laboratory diet. Partial hepatectomy (resection of 65-70 % of the liver tissue) was performed under mild ether anaesthesia by the method of Higgins and Anderson (1931). The technique described by Schreiber and Schreiberová (1957) was chosen for thyroidectomy.

Propylthiouracil (Propycil, Kali-Chemie, FRG) was dissolved in drinking water in a dose of 1 g per litre and the resultant solution served as the sole source of fluids. The rats were given propylthiouracil for three days before partial hepatectomy and thereafter till the end of the experiment. The controls were given drinking water without propylthiouracil throughout the experiment. Triiodothyronine (liothyronine chloride as the preparation Liothyronin,

Gedeon Richter, Hungary) was administered by stomach tube in a dose of $200 \mu\text{g.kg}^{-1}$ body mass under mild ether anaesthesia. The sodium salt of L-thyroxine (Reanal, Hungary), dissolved in saline, was injected intraperitoneally in a dose of 1 mg.kg^{-1} , three times at 24-hour intervals, likewise under mild ether anaesthesia.

Specific DNA activity in the liver was determined by means of ^{14}C -labelled thymidine (Short *et al.* 1969). One hour before the rats were killed (by decapitation), the isotope was injected intraperitoneally in a dose of $22.2 \times 10^5 \text{ Bq.kg}^{-1}$ (specific activity $1.85 \text{ GBq.mmol}^{-1}$). The radioactivity of the samples was measured with a Delta 300 (Nuclear, Chicago, USA) scintillation counter of radioactivity. The DNA content of the liver was determined by means of the diphenylamine reagent (Burton 1956). Using histological liver sections stained with haematoxylin and eosin, the mitotic activity of the liver parenchyma cells (the number of mitoses per 1 000 hepatocytes in two liver tissue sections) was determined in every rat.

The figures and the table give the arithmetical means and the standard errors of the means. The experimental and the control groups each comprised 7–9 rats. An unpaired t-test was used for the statistical evaluation of the results.

Results

In the first part of the experiments, the effect of propylthiouracil on liver regeneration was studied in female rats.

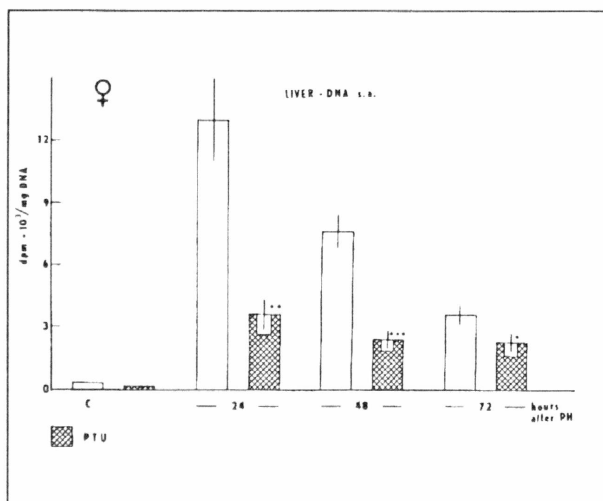


Fig. 1

DNA specific activity ($\text{dpm} \times 10^3/\text{mg DNA}$) before and after partial hepatectomy (PH) in the liver of experimental female rats given propylthiouracil (PTU) and in the control females given none. Mean values \pm the standard errors of the means (7 animals in every group). Statistical significance of differences between the experimental and the control groups; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.01$.

The measurements were carried out 24, 48 and 72 h after partial hepatectomy. Fig. 1 gives the specific DNA activity values characterizing the synthesis of these nucleic acids in the liver. A slight, statistically nonsignificant, decrease in DNA synthesis also occurred in intact, unresected livers after the administration of propylthiouracil. After partial hepatectomy – in keeping with the findings of other authors – a marked increase in DNA synthesis was obtained in the control groups, with maximum values 24 h after the operation. In rats given propylthiouracil, partial hepatectomy was likewise followed by stimulation of DNA synthesis in the liver, but at all the intervals studied the values were significantly lower than in the control groups (at the first two intervals they amounted to roughly 25 % of the control value).

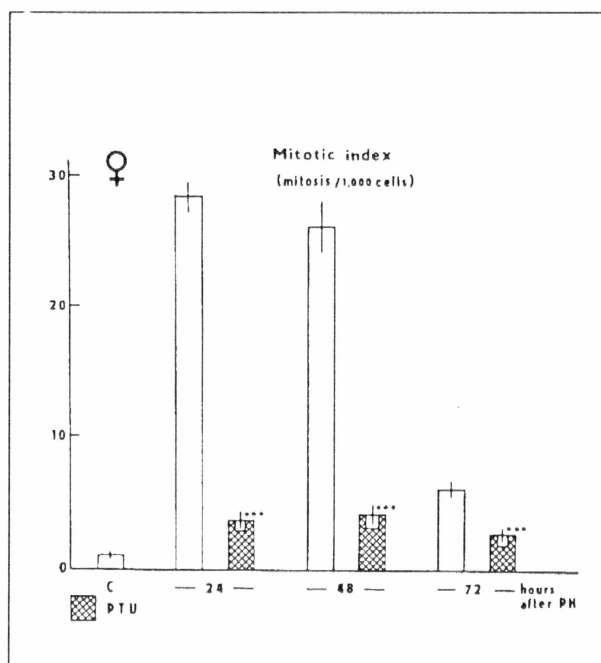


Fig. 2

Mitotic activity of the hepatocytes (the number of mitoses per 1 000 hepatocytes) before and after partial hepatectomy (PH) in experimental female rats given propylthiouracil (PTU) and the control females given none. Mean values \pm the standard errors of the means (7 animals in every group). Statistical significance of differences between the experimental and control groups. *** $p < 0.01$.

Fig. 2 shows the hepatocyte mitotic index in the same groups of rats as in Fig. 1. In rats with an intact liver it does not exceed 1 per thousand. After partial hepatectomy, the number of mitoses in the control groups rose very sharply. As in the case of DNA synthesis, we recorded a significantly smaller number of mitoses in rats treated with propylthiouracil than in the control groups at all the given post-hepatectomy intervals.

In further experiments we tested the effect of propylthiouracil on male rats (Fig. 3).

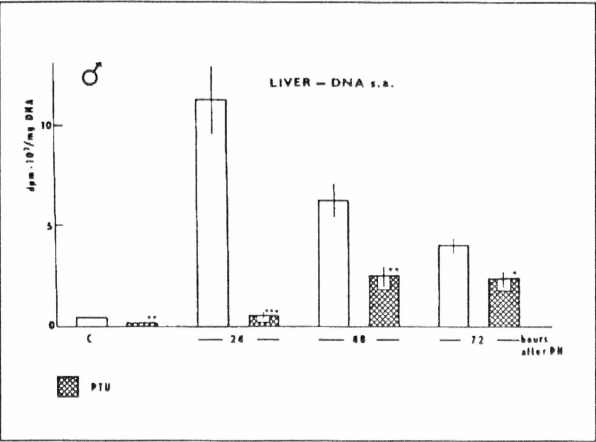


Fig. 3
DNA specific activity (dpm x 10³/mg DNA) before and after partial hepatectomy (PH) in the liver of experimental male rats given propylthiouracil (PTU) and the control males given none. Mean values ± the standard errors of the means (7 animals in every group). Statistical significance of differences between the experimental and control groups; ^X p < 0.05, ^{XX} p < 0.01, ^{XXX} p < 0.001.

As in females, its administration was followed by a decrease in liver DNA synthesis, but in this case the drop was statistically significant. The inhibitory effect of propylthiouracil was also manifested after partial hepatectomy; 48 and 72 h after the operation, the liver DNA synthesis values were comparable to those in females, but at 24 h inhibition of DNA synthesis in propylthiouracil-treated males was significantly greater. The measured values were practically the same as those in rats with an intact liver.

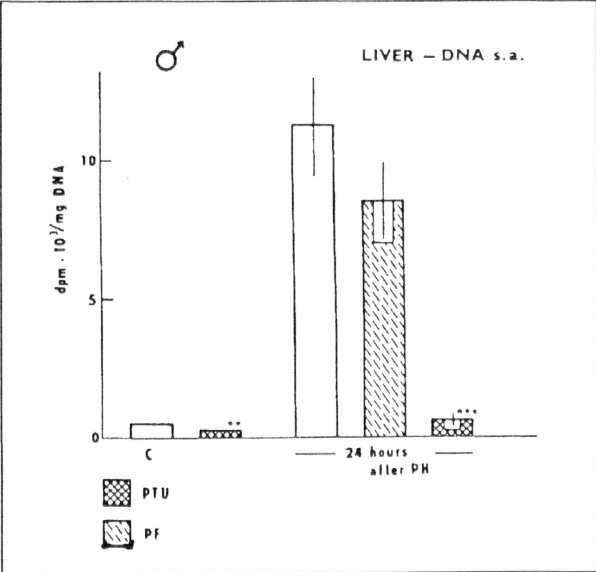


Fig. 4.

DNA specific activity (dpm x 10³/mg DNA) before and after partial hepatectomy (PH) in the liver of male rats fed permanently *ad libitum* on a standard laboratory diet (C), in male rats given propylthiouracil (PTU) and in pair-fed male rats (PF) given food in an amount corresponding to the spontaneous food intake of the rats given PTU. Mean values ± the standard errors of the means (8 animals in every group). Statistical significance of differences in relation to the control group (fed *ad libitum*): ^{XX} p < 0.01, ^{XXX} p < 0.001.

During the experiments, the body mass of rats given propylthiouracil for three days fell by 11 % of the initial values, whereas in the controls, fed three days on the laboratory diet, it rose by 9.5 % on the average. We therefore proceeded to determine food consumption (Tab. 1). The rats were kept singly in cages with a mesh floor without any bedding. On all three days before the operation, the food intake of propylthiouracil-treated rats was statistically significantly lower than that of the controls. After partial hepatectomy, when food intake also fell markedly in the control rats, food consumption in the two groups was practically the same. Since marked restriction of food intake has a partly inhibitory effect on liver DNA synthesis (well-known from the literature and also confirmed by the results of our preceding experiments), we were interested in how far propylthiouracil and how far energy restriction was responsible for the decrease. In the next experiments, the rats were therefore kept singly and were pair-fed, i.e. during the experiment they were given only as much food as was consumed spontaneously by the rats given propylthiouracil. In rats fed in this manner, liver DNA synthesis 24 h after partial hepatectomy was lower than in the controls fed continuously *ad libitum*, but was many times higher than in animals given propylthiouracil.

The results of the first part of the experiments clearly demonstrated a marked inhibitory effect of propylthiouracil on the initiation of liver regeneration. However, since it was not clear whether this was due to inhibition of thyroid function, or whether propylthiouracil affected the liver tissue directly, we undertook further experiments in which the initiation of liver regeneration was influenced by thyroidectomy, alone or combined with the administration of propylthiouracil. In some rats, propylthiouracil was combined with the administration of thyroid hormones.

In male rats with an intact thyroid, propylthiouracil caused DNA synthesis in the intact liver tissue to fall, despite the simultaneous repeated administration of thyroxine (Fig. 5). DNA synthesis in the intact liver likewise fell mildly after isolated thyroidectomy, but if propylthiouracil was administered at the same time the decrease was statistically significant. In these rats also, the repeated administration of thyroxine failed to raise liver DNA synthesis to the level of the control group.

Table 1

	Spontaneous food intake (g/kg) male			
	1st day	2nd day	3rd day	4th day (24 h after PH)
C	95.4±5.2	93.6±3.8	93.1±3.2	24.5±3.1
PTU	30.5±4.1***	39.1±4.4***	48.2±5.2***	22.8±3.3

Consumption of the standard laboratory diet (g/kg body weight) before and after partial hepatectomy (PH) by the control male rats and males given propylthiouracil (PTU). Mean values ± the standard errors of the means (7 animals in every group). Statistical significance of differences in relation to the control group: xxx p < 0.00

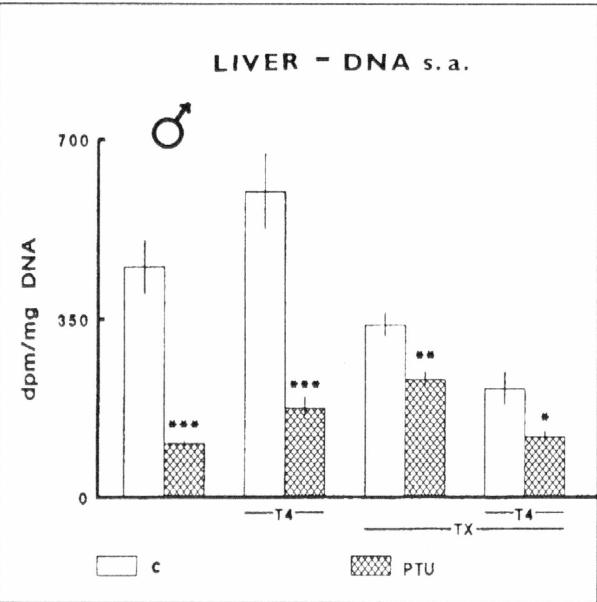


Fig. 5
DNA specific activity (dpm/mg DNA) in the intact liver of male rats given propylthiouracil (PTU) under conditions of hypothyroidism induced by thyroidectomy (TX) and compensated by the i.p. administration of thyroxine (T₄) in 3 doses of 1 mg/kg at 24 h intervals. Mean values ± the standard errors of the means (7 animals in every group). Statistical significance of differences in relation to the control group: ^x p < 0.05, ^{xx} p < 0.01, ^{xxx} p < 0.001.

Thyroidectomy itself did not inhibit the increase in DNA synthesis measured 24 h after partial hepatectomy (it was only slightly smaller than in the control group of euthyroid animals – Fig. 6). Thyroidectomy combined with the administration of propylthiouracil led to statistically highly significant (p < 0.001) inhibition of DNA synthesis, which was not prevented by the simultaneous administration of triiodothyronine.

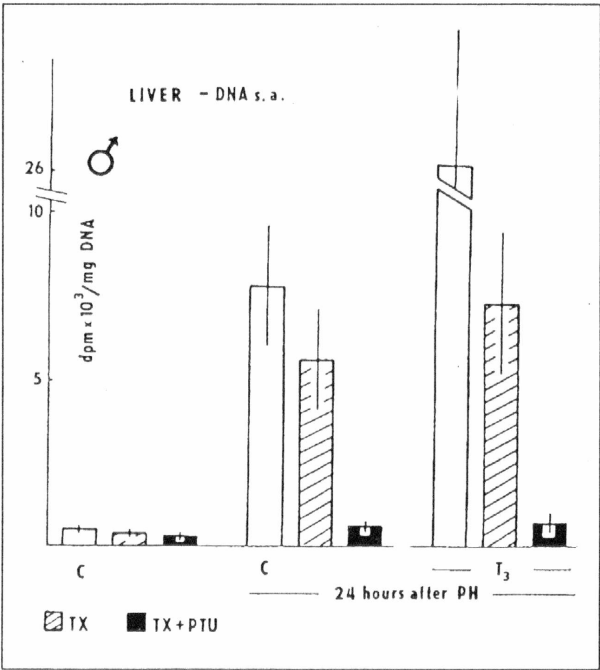


Fig. 6
DNA specific activity (dpm x 10³/mg DNA) 24 h after partial hepatectomy (PH) and a single i.g. dose of triiodothyronine (T₃) (200 µg/kg) in the control male rats and in males with hypothyroidism induced by thyroidectomy (TX), alone or combined with the administration of propylthiouracil (PTU). Mean values ± the standard errors of the means (9 animals in every group).

Discussion

Our experiments demonstrated that propylthiouracil had a pronounced inhibitory effect on DNA synthesis in intact and regenerating liver in both euthyroid and thyroidectomized rats and that not even thyroid hormone substitution blocked it. It is clear from the results that the effect of propylthiouracil on liver

tissue is a direct one and that it is not mediated by the effect on the thyroid. The mechanism of this direct effect is difficult to define exactly, however. The thyroid hormones are taken up by the hepatocytes, where triiodothyronine is bound to a receptor in the nucleus and then, specifically, to the non-histone protein in the nuclear chromatin (Knopp and Brtko 1989). The hormone-receptor complex causes gene expression and specific messenger RNA, ribosomal RNA and finally specific proteins (enzymes) are formed. Propylthiouracil is known to delay the conversion of thyroxine to triiodothyronine and also of reverse triiodothyronine (rT₃) to 3,3'-diiodothyronine in liver tissue (Cavalieri and Pitt-Rivers 1981). It is thus evident that the effect of the thyroid hormones on DNA synthesis in the liver is severely impaired by propylthiouracil at the hepatocyte level. Of course, it is not known whether the above reactions are the sole or the main site of the interference of propylthiouracil with the thyroid hormones in the hepatocyte. The participation of nutritional alteration, recorded in our experiments, also indicates that further (in particular metabolic) factors induced by the administration of propylthiouracil may have a bearing on DNA synthesis in the liver. In this connection an effect on the pentose cycle, adenylate cyclase activity and the glutathione level could be taken into consideration, for instance. Raheja *et al.* (1984), who studied the influence of propylthiouracil on the hepatotoxicity of acetoaminophen, also drew attention to the significance of the nutritional factor in an analysis of its effects. If we take all the above considerations into account, we come to the conclusion that marked propylthiouracil-induced impairment of liver DNA synthesis is most likely the outcome of a complex effect on the hepatocytes, involving interference with both hormonal and nutritional processes.

The finding that inhibition of liver DNA synthesis after the administration of propylthiouracil was greater in males than in females can be attributed to differences in their hormonal equipment. As demonstrated by Francavilla *et al.* (1984, 1989), partial hepatectomy is followed by elevation of the blood oestrogen concentration and of the number of oestrogen receptors in the liver tissue. Oestrogens stimulate DNA synthesis in the liver after partial hepatectomy and their positive effect on regeneration probably partly compensates the inhibitory effect of propylthiouracil.

The direct effect of propylthiouracil on liver tissue is also documented by the results of Cooper *et al.*

(1984), who found that a single dose afforded protection against galactosamine-induced liver damage without the thyroid hormone level being affected; other antithyroid thiols had no such protective effect. It is clearly impossible to regard the lower sensitivity of the liver to a noxa after the administration of propylthiouracil automatically as the outcome of propylthiouracil-induced hypothyroidism without verifying thyroid hormone levels, as Schweden *et al.* (1986) had done. Propylthiouracil was also shown to have a hepatoprotective effect in the case of liver damage induced by acetoaminophen (Yamada *et al.* 1981) and alcohol (Israel *et al.* 1975). The mechanism of this protective effect has not been adequately elucidated, however. In the case of acetoaminophen liver damage, the possible substitution of propylthiouracil for glutathione has been considered, since in acetoaminophen-induced liver injury glutathione has a protective effect, but its concentration in the liver falls during damage by this hepatotoxic substance (Mitchell *et al.* 1973). Propylthiouracil is employed clinically in the treatment of alcohol-induced liver damage associated with hypermetabolism (Orrego *et al.* 1987). On the basis of the experimental results presented here, it can be stated that, in the clinical use of propylthiouracil, one must also reckon with its direct effect on the liver tissue. As regards its clinical utilization, the extent of its effect on hepatocytes requires further experimental analysis.

In conclusion, the role of the thyroid hormones in the mechanism of liver regeneration should be briefly mentioned. On the basis of experiments in which the thyroid hormones, in the hepatocytes of the intact liver (Lee *et al.* 1968) and of the liver remaining after partial hepatectomy (Červinková *et al.* 1984), stimulated both DNA synthesis and mitotic activity, it can be presumed with certainty that these hormones participate in the above mechanism. However, from the small decrease in the regenerative activity of the liver found in thyroidectomized rats by Short *et al.* (1980a) as well as by ourselves, it can be concluded that *in situ*, under normal conditions, the thyroid hormones do not play a decisive role in stimulating the onset of liver regeneration. This effect is evidently the outcome of the complex action of a series of factors whose interplay in assuring the initiation and development of liver regeneration is the subject of intensive study (Michalopoulos 1990).

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Reprint Requests

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