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# Decreased synthesis of DNA in regenerating rat liver after the administration of reserpine

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## Summary

1. Reserpine given to rats before the enhanced synthesis of DNA begins 14h after partial hepatectomy markedly depresses thymidine uptake into DNA at 24 hours.

2. At this time decreased activity of liver thymidine kinase but unchanged thymidine 5′-nucleotidase were observed.

3. Reserpine has no effect on DNA synthesis when administered simultaneously with the labelled thymidine 2 h before killing.

4. With depressed DNA synthesis after reserpine administration there is no significant decrease of liver RNA synthesis.

## Introduction

Regenerating liver has a population of cells with high mitotic activity and elevated synthesis of DNA (Bollum & Potter, 1959; Bucher, Swaffield & Di Troia, 1964). The model is convenient for studies of the action of drugs which affect DNA synthesis in eukaryotic systems (Hurwitz & Carter, 1969; Witschi, 1970; Lea, Sasovetz, Musella & Morris, 1970; Čihák & Veselý, 1972). Recently we have found a decreased synthesis of DNA in regenerating liver of rats subjected to spinal cord C7 transection after partial hepatectomy (Vaptzarova, Popov, Ceselý & Čihák, 1973). In this paper we describe the effect of reserpine on the synthesis of DNA in the liver. Reserpine causes the depletion of noradrenaline from sympathetic ganglia and fibres resulting in the decrease of peripheral sympathetic activity (Muscholl & Vogt, 1958). The inhibition of DNA synthesis after reserpine indicates a new site of its action and may explain the antileukaemic effect of this drug (Goldin, Burton, Humphreys & Venditti, 1957; West, Baird, Steward & Pradhan, 1961).

## Methods

### *Animals and cell-free liver extracts*

Groups of 4–6 male albino rats (170–185 g) were used. Experiments were started between 8–10 a.m. and partial hepatectomy (66%) or sham-operation were performed under light ether narcosis (Higgins & Anderson, 1931). Drugs were injected i.p. and controls received the same volume of 0·9% w/v NaCl solution. The animals were killed by cervical dislocation, bled and the excised livers were homogenized after cooling in a glass homogenizer with a tight-fitting Teflon pestle with 3 volumes of 0·025 M Tris-HCl buffer (pH 7·5) containing $2{\cdot}5 \times 10^{-3}$ M KCl and 5 mM

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$Mg^{2+}$ ions. Homogenates were used for the estimation of RNA and DNA synthesis and supernatant fractions (10,000 rev/min, 20 min, 2° C) were used as a source of enzyme activity.

### *Assay of enzyme activities*

Thymidine kinase was assayed in 0·05 Tris-HCl buffer (pH 7·8) during a 10 min incubation period at 37° C with $5\times10^{-5}$M thymidine-2-[$^{14}$C], $3\times10^{-3}$M adenosine 5′-triphosphate and $1{\cdot}5\times10^{-3}$M $Mg^{2+}$ ions as described by Čihák & Veselý, 1972. Thymidine 5′-nucleotidase was measured at a lower pH of the incubation mixture (Fritzson, 1967) during a 10 min incubation period at 37° C in 0·04 M sodium acetate buffer (pH 6·0) with $5\times10^{-5}$M thymidine-2-[$^{14}$C] 5′-monophosphate and 0·1 ml of liver extract corresponding to 25 mg of liver in a total volume of 0·5 ml. Aliquots of incubation mixtures withdrawn during the linear course of the respective enzyme reactions were separated chromatographically on a Whatman paper No. 1 in different solvent systems as described by Čihák & Veselý, 1972. Corresponding chromatographic spots located according to standards were cut out and their radioactivity was measured with a Packard liquid scintillation spectrometer. The activity of enzymes is expressed as nmol of the product formed during a 10 min incubation period.

### *Measurement of hepatic DNA and RNA synthesis*

Orotic-6-[$^{14}$C] acid (0·5 $\mu$Ci/0·2 $\mu$mol) and thymidine-2-[$^{14}$C] (1·0 $\mu$Ci/0·3 $\mu$mol) were injected i.p. in 0·3 ml at different time intervals after partial hepatectomy and 2 h before killing. Liver homogenates were repeatedly extracted with cold 0·2 M $HClO_4$ to remove acid-soluble radioactive compounds. The isolation of spectroscopically pure uridine-2-[$^{14}$C] 2′(3′)-phosphate after alkaline hydrolysis of RNA (1 M KOH, 18 h, 20° C) and of pure thymine-2-[$^{14}$C] after acid hydrolysis of DNA (70% $HClO_4$, 1 h, 100° C) has been carried out as described by Čihák & Brouček, 1972. The utilization of radioactive precursors is expressed as the specific radioactivity of isolated compounds in d/min per micromole.

### *Drugs*

Adenosine 5′-triphosphate and thymidine 5′-monophosphate were obtained from Calbiochem, Luzerne. Reserpine was a product of VEB Arzneimittelwerk, Dresden. Orotic-6-[$^{14}$C] acid (48 mCi/mmol), thymidine-2-[$^{14}$C] and thymidine-2-[$^{14}$C] 5′-monophosphate (44 mCi/mmol) were provided by the Institute for Research, Production and Uses of Radioisotopes in Prague.

## Results

In our previous studies we were interested in the effect of different drugs and factors on the incorporation of thymidine into DNA in regenerating rat liver (Čihák & Veselý, 1972 ; Vaptzarova *et al.*, 1973), and we now describe the marked depression of hepatic DNA synthesis by reserpine. The maximal effect in 24 hour regenerating liver was obtained when the drug was administered before the exponential increase of DNA synthesis, i.e. up to 14 h after hepatectomy (Fig. 1). In the liver of sham-operated animals no similar decrease of DNA synthesis was observed. Reserpine had no effect when administered shortly before killing of animals (22 h

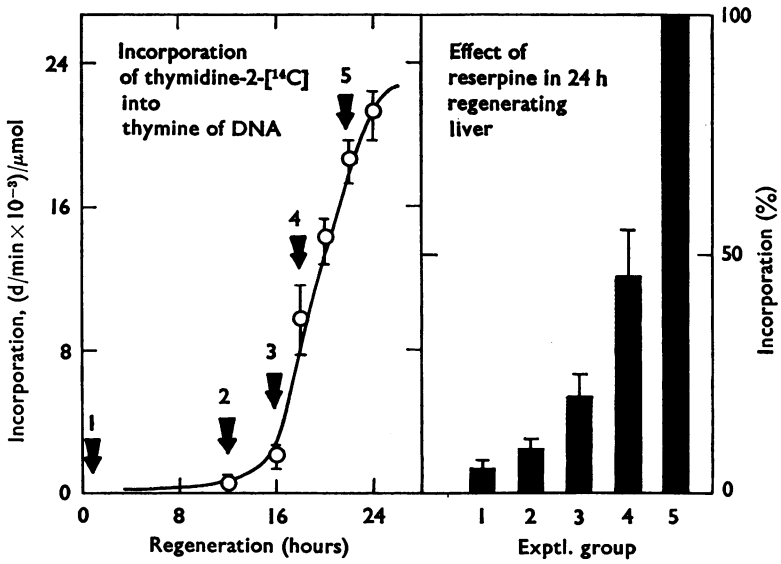


FIG. 1. Depressed DNA synthesis in regenerating rat liver after reserpine administration. Groups of 4–6 male rats (175 g) were injected i.p. at different time intervals after partial hepatectomy with reserpine (2·5 mg/kg) as indicated by arrows (1–5). Left—increase of DNA synthesis during liver regeneration measured by the rate of thymidine-2-[$^{14}$C] (1 $\mu$Ci/0·3 $\mu$mol per animal) incorporation during a 2 h pulse; right—effect of reserpine administered at different stages of the regeneration process on the rate of DNA synthesis in 24 h regenerating liver expressed as percentage of untreated regenerating controls (=100%).

after operation), and simultaneously with the labelled thymidine. Synthesis of RNA even when DNA formation was depressed by reserpine was unchanged or slightly increased (Table 1).

TABLE 1. *Effect of reserpine on DNA and RNA synthesis in regenerating rat liver*

| Nucleic acid | Control (d/min)/μmol±S.E. | Reserpine-treated (d/min)/μmol±S.E. |
|---|---|---|
| Sham-operated | | |
| DNA | 410± 56 | 335± 42 |
| RNA | 2,400± 420 | 3,030±475 |
| Regenerating | | |
| DNA | 20,550±2,630 | 2,860±920 |
| RNA | 6,950± 730 | 7,715±820 |

Groups of 3-5 rats were injected i.p. with reserpine (5 mg/kg) or saline 14 h after operation and 10 h before killing. The livers were excised and assayed individually. Specific radioactivity of thymine-2-[$^{14}$C] and uridine-2-[$^{14}$C] 2′ (3′)-phosphate isolated from liver DNA and RNA, respectively is expressed as (d/min)/μmol. Typical data from three independent experiments are presented.

TABLE 2. *Decreased thymidine phosphorylation in cell-free liver extract after reserpine treatment*

| Animals | Newly formed (nmol) | | Inhibition (%) |
|---|---|---|---|
| | TTP+TDP | TMP | |
| Control regenerating | 1·97; 2·41 | 10·30; 14·45 | –; – |
| Reserpinized | 1·14; 1·81 | 5·53; 7·13 | 45·7; 47·0 |

Reserpine (2·5 mg/kg) was injected i.p. 12 h after partial hepatectomy and 12 h before killing to groups of 3 animals. $5\times10^{-5}$M thymidine-2-[$^{14}$C], $3\times10^{-3}$M ATP and $1{\cdot}5\times10^{-3}$M $Mg^{2+}$-ions were incubated for 10 min at 37° C with cell-free liver extracts corresponding to 25 mg of the liver and incubation mixtures were analysed by paper chromatography. Results of two independent experiments are presented.

In an effort to explain the decrease of DNA synthesis and to exclude possible alterations in thymidine permeation, an analysis of radioactive compounds in the intracellular acid-soluble liver pool after thymidine-2-[$^{14}$C] i.p. administration was undertaken. Simultaneously metabolic conversion of thymidine in the cell-free liver extract was followed. The level of newly formed thymidine 5′-phosphates in the liver *in vivo* was lower (about 20%) in reserpine-treated animals. This correlates with thymidine phosphorylation in liver extracts *in vitro* (Table 2). Twelve hours after the administration of reserpine, the synthesis of thymidine 5′-monophosphate was thus significantly depressed both *in vivo* and in liver extract *in vitro*.

Measurements of the activity of enzymes responsible for the metabolic conversion of thymidine revealed an impressive decrease of thymidine kinase compared to its activity in the control 24 h regenerating liver (Table 3). The decrease is not due to the change in the activity of thymidine 5′-nucleotidase, an enzyme which catalyses in unpurified cell-free liver extract the degradation of the newly formed thymidine 5′-monophosphate.

TABLE 3. *Synthesis and degradation of thymidine 5′-monophosphate in regenerating rat liver after reserpine administration*

| Administered | TdR-kinase nmol | TMP-nucleotidase nmol |
|---|---|---|
| Sham operated | | |
| Control | 4·02; 4·12 | 39·8; 44·6 |
| Reserpine | 3·74; 3·58 | 41·2; 40·7 |
| Regenerating | | |
| Control | 12·02; 10·63 | 31·0; 34·5 |
| Reserpine | 5·51; 5·48 | 28·8; 32·3 |

Reserpine (5 mg/kg) or saline were administered i.p. to groups of 3 rats 14 h after partial hepatectomy and 10 h before killing. Activity of enzymes thymidine kinase (TdR-kinase) and thymidine 5′-nucleotidase (TMP-nucleotidase) was measured as described in Methods. Results of two independent experiments are given.

## Discussion

It was suggested by Khazan, Sulman & Winnick (1961) and Westermann, Maickel & Brodie (1962) that reserpine stimulates transitorily the pituitary-adrenal cortex axis which could explain the effect of reserpine on DNA synthesis in regenerating rat liver reported here. However, DNA synthesis in regenerating liver is regulated by a number of different phenomena and mechanisms (Barbiroli & Potter, 1971). The inhibitory effect of glucocorticoid hormones on DNA synthesis in both intact and regenerating rat liver has been observed by many authors (e.g. Rizzo, Heilpern & Webb, 1971; Henderson, Fischel & Loeb, 1971) and the maximal effect was achieved when corticosteroids were administered 15–20 h after hepatectomy (Raab & Webb, 1970).

However, under our experimental conditions reserpine (2–6 mg/kg) depressed DNA synthesis in regenerating rat liver about 80%, whereas hydrocortisone (2–10 mg/kg) under identical conditions depressed it by only about 20–40%. Against the idea that the action of reserpine on DNA synthesis is mediated through the activation of pituitary-adrenal cortex axis is also the observation that adrenocorticotrophic hormone does not inhibit liver regeneration (Moolten, Oakman & Bucher, 1970). Recently a study dealing with the effect of reserpine on the incorporation of thymidine into mouse liver DNA has appeared (Hach, Mitznegg &

Heim, 1972). The experimental evidence excludes the possibility that the effect of reserpine on DNA synthesis could be explained by the anorexia and hypothermia induced by this drug. Further experiments are thus required to explain the molecular mechanism of the inhibition of hepatic DNA synthesis observed after the administration of reserpine.

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