Increased Synchrony of DNA Synthesis in Regenerating Rat Liver after Continuous Infusion of Hydroxyurea

Regenerating rat liver after partial hepatectomy is a standard model for studies on growth regulation in vivo[1]. The main disadvantage, however, for quantitative biochemical investigations is the fact that DNA synthesis after partial hepatectomy proceeds in an only partially synchronized pattern[2–4]. Autoradiographic studies during early periods of liver regeneration revealed an influx rate of hepatocytes into DNA synthesis of 6.31%/h from 18.00 to 19.00 h, and of 11.39%/h from 19.00 to 20.00 h after partial hepatectomy[5]. Maximum number of hepatocytes simultaneously in DNA synthesis does not exceed 30 to 40% (depending on age of animals[6]) at any time after $^2/_3$ hepatectomy[4].

To construct a regenerating liver model with higher synchrony of DNA synthesis, stimulus for DNA synthesis by partial hepatectomy was combined with prolonged inhibition of start of DNA synthesis. Hydroxyurea (HU) has been used as an effective inhibitor of nuclear, but not of cytoplasmic[7,8] DNA synthesis in vivo[9,10] and in vitro[11–13]. Administration of HU to partially hepatectomized rats resulted in a reversible inhibition of DNA synthesis[14–16] without measurable effects on RNA and protein synthesis. Hepatotoxicity has been found to be low[17]. There are no reports on the degree of synchronization of DNA synthesis in partially hepatectomized rats which received a continuous infusion of HU. In contrast to a single application, continuous infusion of HU leads to subtotal synchrony of start of DNA synthesis.

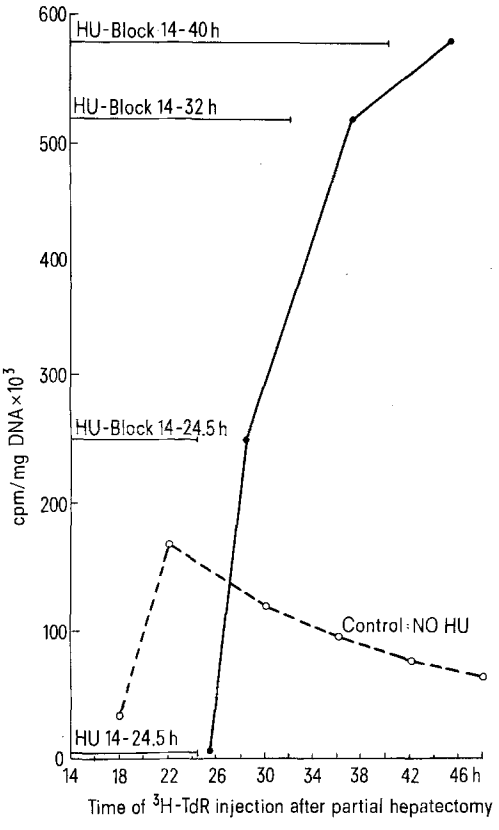

Fig. 1. Increase of specific activity of DNA in liver of partially hepatectomized rats after continuous infusion of hydroxyurea (1.25×10^{-3} M kg^{-1} h^{-1}) as a function of block duration and interval between discontinuation of HU and injection of ^{3}H-thymidine. Control animals (open squares) are partially hepatectomized, but did not receive HU. Abscissa: Time of injection of ^{3}H-thymidine (0.8 μCi g^{-1}). Rats sacrificed 60 min thereafter. Ordinate: Specific activity of DNA as determined according to [20,21].

Materials and methods. Male Wistar rats (220–240 g) were partially hepatectomized according to HIGGINS[18]. At 14 h after operation, i.e. prior to start of DNA synthesis at 15 to 18 h under these experimental conditions[19], the animals received a single i.v. injection of HU at a dose of 1.69×10^{-3} Mkg^{-1}, dissolved in saline solution, immediately followed by a continuous infusion of HU at a dose of 1.25×10^{-3} Mkg^{-1}h^{-1} via tail vein for periods up to 26 h. At different intervals after discontinuation of the drug, rats were injected with ^{3}H-thymidine i.p. (0.8 μCi g^{-1}, specific activity 5 Ci/mM, Radiochemical Centre Amersham) and were sacrificed 60 min later. Hepatectomized rats without HU infusion served as controls. After extraction of DNA from the liver according to MUNRO and FLECK[20], DNA content of aliquots of the extract was determined according to BURTON[21] and radioactivity was measured in a liquid scintillation counter (Packard Tricarb 3214). Autoradiographs were prepared from 4 μm sections of paraffin embedded liver tissue using Kodak AR 10 stripping film. After exposure for 21 days, the autoradiographs were developed and stained with H & E.

Results and discussion. Inhibition of DNA synthesis in hepatocytes of regenerating rat liver is reversed after termination of HU treatment. The interval between stopping HU infusion and start of DNA synthesis depends on the duration of HU treatment. Maximum augmentation of specific activity of DNA is observed 4 to 7 h after withdrawal of the drug. Peaks of specific activity of DNA, after release from HU block, increase as a function

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of block duration as shown in Figure 1. They exceed the average values of hepatectomized control rats without HU infusion by factors up to about 7.

Previous studies on the degree of synchrony of DNA synthesis after partial hepatectomy revealed that hepatocytes start DNA synthesis in a topographically determined sequence[4]. At 20 to 24 h after operation, the maximum of labelled hepatocytes 60 min after injection of tritiated thymidine is found in the periportal zone of the liver lobule, at 34 h in the intermediary zone, and at 40 h after hepatectomy in parts surrounding the hepatic vein[4]. ^{3}H-thymidine autoradiographs prepared during continuous infusion of HU and at different intervals after termination of HU infusion show that hepatocytes are accumulated at the G_1-S boundary and start, after release from HU block, DNA synthesis with a considerably higher degree of synchrony as compared with normal regenerating liver. An example is given in Figure 2. HU infusion from 14 to 40 h after partial hepatectomy and sacrifice of the rat at 45 h after operation, 60 min after injection of tritiated thymidine, results in a labelling index of 90%. Only a small fringe of tissue around terminal hepatic venules contains unlabelled hepatocytes.

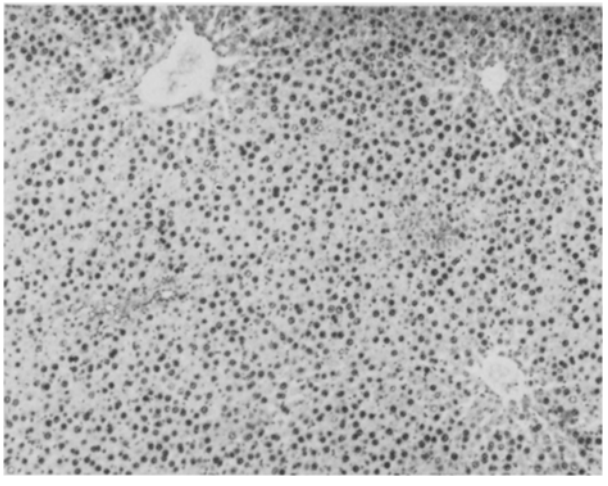

Fig. 2. Increased synchrony of hepatocellular DNA synthesis after release from hydroxyurea block. Autoradiogram of rat liver after continuous infusion of HU (1.25×10^{-3} M kg^{-1} h^{-1}) from 14 to 40 h after partial hepatectomy. Injection of ^{3}H-thymidine (0.8 μCi g^{-1}) at 44 h, sacrifice of the rat at 45 h after liver resection. Stripping film Kodak AR 10, exposure time 21 days, H & E staining. ×100.

Subtotal synchrony of DNA synthesis observed in the liver after this mode of a continuous infusion of HU has not been obtained before by any other method in a cell system in vivo. This model of a highly synchronized, differentiated normal parenchymal cell population might be a useful tool for biochemical investigations in vivo of events essential for G_1-S transition. Furthermore, the synchrony of DNA synthesis provides an excellent opportunity for studies on interactions between carcinogens and replicating DNA in vivo (manuscript in preparation).

Zusammenfassung. Intravenöse Dauerinfusion von Hydroxyharnstoff bis zu 40 h nach partieller Hepatektomie inhibiert den Beginn der DNA-Synthese in der Rattenleber. Nach Aufhebung des Blocks treten Hepatozyten subtotal synchronisiert in die DNA-Synthese ein. Ein ähnlich hoher Synchronie-Grad wurde bisher in vivo nicht erreicht.

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Influence of the Anti-Typhoparatyphoid Vaccines on the in vivo Activity of the Glucosaminyl-Transferases of Animals Infected by Myxovirus

Preceding work has shown that the infection of mice by myxovirus influenza leads to the hyperactivity of splenic and hepatic microsomal glycosyl-transferases, in vivo or in vitro, in acellular system. Under these conditions it seemed worthwhile to seek the in vivo influence of antibacterial and antiviral vaccinations, whether followed or not by viral infection, on these glycosylation activities of the proteins. The results previously obtained with the antipoliomyelitic vaccine have shown the important modifications at the level of the serum and of the soluble cytoplasmic stage of the infected animals hepatocytes[1,2]. The aim of this work is to show the influence on the rate of the glycosylations, of an anti-bacterial vaccine administered alone or before the influenza infection.

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Table I. Experimental conditions of vaccination and viral infection for the 5 lots of animals T, A, B, C, D

Lots of animals	Programs
T	^{14}C-glucosamine ← 4 h → decapitation
A	TAB ← 48 h → ^{14}C-glucosamine ← 4 h → decapitation
B	TAB ← 48 h → influenza ← 48 h → ^{14}C-glucosamine ← 4 h → decapitation
C	TAB ← 96 h → influenza ← 48 h → ^{14}C-glucosamine ← 4 h → decapitation
D	influenza ← 48 h → ^{14}C-glucosamine ← 4 h → decapitation