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Increased glycosylation capacity in regenerating rat liver is paralleled by decreased activities of CMP-*N*-acetylneuraminate hydrolase and UDP-galactose pyrophosphatase

Willem VAN DIJK, Anne-Marie LASTHUIS, Leo A. W. TRIPPELVITZ and Hans G. MUILERMAN
Department of Medical Chemistry, Faculty of Medicine, Vrije Universiteit, 1007 MC Amsterdam, The Netherlands

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In regenerating rat liver the activities of CMP-*N*-acetylneuraminate hydrolase and UDP-galactose pyrophosphatase were decreased to 40–50% of control values within 35 h after partial hepatectomy. In the same time period the activities of sialyltransferase and galactosyltransferase were increased, and the initial sharp decrease in the carbohydrate content of liver and serum glycoproteins was largely restored. The antiparallel nature of these events is suggestive of an involvement of nucleotide-sugar-hydrolysis enzymes in rat liver glycoprotein biosynthesis.

The physiological function of nucleotide-sugar-hydrolysing enzymes was hitherto unknown. An indication that these enzymes might be involved in glycoprotein biosynthesis was obtained by Jato-Rodriguez *et al.* (1976) and Nelson *et al.* (1977). They have reported that, during the oestrous cycle of the rat, UDP-galactose pyrophosphatase activity in the endometrium is varied in an antiparallel way with respect to UDP-galactose:glycoprotein galactosyltransferase activity and glycoprotein production in the endometrium. We were interested to see whether a general relationship exists between the activities of nucleotide-sugar-hydrolysing enzymes and glycoprotein biosynthesis. It has been reported that, in regenerating rat liver after partial hepatectomy, the activities of a variety of glycosyltransferases are increased (Bauer *et al.*, 1976; Khalkhali *et al.*, 1977; Serafini-Cessi, 1977; Ip, 1979), the nucleotide-sugar concentrations are varied (Bauer *et al.*, 1976; Harms *et al.*, 1973; Okuba *et al.*, 1977) and the biosynthesis of glycoproteins is altered (Okamoto & Akumatsu, 1977, 1980). Therefore regenerating liver was used by us to study the possible relationship described above.

Experimental

Treatment of animals

Male Wistar rats (240–295 g) were fed *ad libitum* with normal laboratory chow in a room with constant temperature with 12 h each of light (07:00–19:00 h) and darkness. Rats were partially hepatectomized or sham-operated by the method of Higgins & Anderson (1931). The operations were carried out between 08:45 and 11:45 h. Up to 12 h after surgery, rats were given a solution of 20% (w/v) glucose *ad libitum* instead of drinking water. The excised liver lobes were frozen and stored in liquid N_2 until used as controls (see the legend to Fig. 1 below). For each time point, three rats were used, which were treated simultaneously and were housed in the same cage.

Preparation of rat serum and liver homogenates

At various times after operation (0–189 h) blood was obtained by cardiac puncture and was allowed to clot. The sera were stored at −40°C until used. Directly after cardiac puncture the rats were killed by decapitation and the livers were perfused *in situ* with phosphate-buffered saline (0.136 M-NaCl/2.6 mM-KCl/8.3 mM-Na_2HPO_4/1.4 mM-KH_2PO_4, pH 7.4) until blanched, whereafter they were removed and were stored in liquid N_2. Homogenates (16%, w/v) were prepared in 0.25 M-sucrose after thawing and mincing of the livers (Van Dijk *et al.*, 1977). One part was used directly for the assay of the activities of nucleotide-sugar-hydrolysing enzymes and of protein. The other part was stored frozen at −20°C until used for the assays of carbohydrate and of the activities of glycosyltransferase and CMP-*N*-acetylneuraminate synthetase.

The particulate fraction of liver was prepared by centrifugation of rat liver homogenate at 100000 *g* for 90 min. Intravesicular materials were removed by washing the pellet once with distilled water.

Assays

The following enzyme activities were assayed essentially by methods described previously: CMP-*N*-acetylneuraminate synthetase (Van Dijk *et al.*, 1973), CMP-*N*-acetylneuraminate hydrolase and UDP-galactose pyrophosphatase (Van Dijk *et al.*, 1981), phosphodiesterase I (Van Dijk *et al.*, 1977) and CMP-*N*-acetylneuraminate:desialylated α_1-acid glycoprotein sialyltransferase (Van Dijk *et al.*, 1979*a*). UDP-galactose:ovalbumin galactosyltransferase activity was assayed at pH 6.0 as described by Berger *et al.* (1978). The glycosyltransferases were incubated at 37°C for 30min, during which time no interference of nucleotide-sugar-hydrolysing enzymes was observed, since over that period the reaction rates appeared to be linear with time (results not shown). Protein was determined by the Coomassie Blue method (Bradford, 1976), with horse serum albumin as a standard. Carbohydrates were determined by g.l.c. (Van Dijk *et al.*, 1979*b*) after methanolysis as described by Clamp *et al.* (1972) and removal of fatty acid methyl esters with n-hexane. *N*-Acetylgalactosamine and fucose were not detectable. In serum, *N*-acetylneuraminate was determined as described below.

Bound *N*-acetylneuraminate and free *N*-acetylneuraminate + CMP-*N*-acetylneuraminate were determined by using the isotope-dilution technique. For the assay of free *N*-acetylneuraminate + CMP-*N*-acetylneuraminate, frozen homogenate or serum was thawed and [^{14}C]*N*-acetylneuraminate (50000d.p.m.) and ethanol [−20°C; up to 70% (v/v)] were added. After centrifugation the supernatant was applied to a column (3.5cm × 0.5cm) of Dowex AG 1 resin (Bio-Rad; X8, Cl^- form, 200–400 mesh) and the metabolites were eluted with 6ml of 30mM-HCl and subsequently assayed as described by Ferwerda *et al.* (1981). Bound *N*-acetylneuraminate in serum was determined as total *N*-acetylneuraminate as described by the same authors. No correction was needed for the amount of free *N*-acetylneuraminate + CMP-*N*-acetylneuraminate, since in serum no free *N*-acetylneuraminate + CMP-*N*-acetylneuraminate could be detected.

Materials

CMP-*N*-acetylneuraminate was prepared as described by Van Dijk *et al.* (1981). CMP-[4,5,6,7,8,9-^{14}C]*N*-acetylneuraminate (sp. radioactivity 1.6Ci/mol) and UDP-[U-^{14}C]galactose (sp. radioactivity 274Ci/mol; diluted to 0.45Ci/mol before use) were obtained from New England Nuclear Corp. [*acetyl*-^{14}C]*N*-Acetylneuraminate (sp. radioactivity 7.2Ci/mol) was kindly given by Dr. W. Ferwerda (Department of Medical Chemistry, Vrije Universiteit, Amsterdam). Ovalbumin (grade V) and UDP-galactose were purchased from Sigma. All other chemicals were obtained from commercial sources and were of analytical grade.

Results and discussion

Shortly after partial hepatectomy the glycosylation capacity appeared to be increased in regenerating rat liver. This is illustrated by the elevated activities of glycoprotein sialyltransferase and galactosyltransferase (Fig. 1). An increased glycosylation capacity seems to be required for the restoration of the protein-bound carbohydrate content in liver membranes and serum, which was

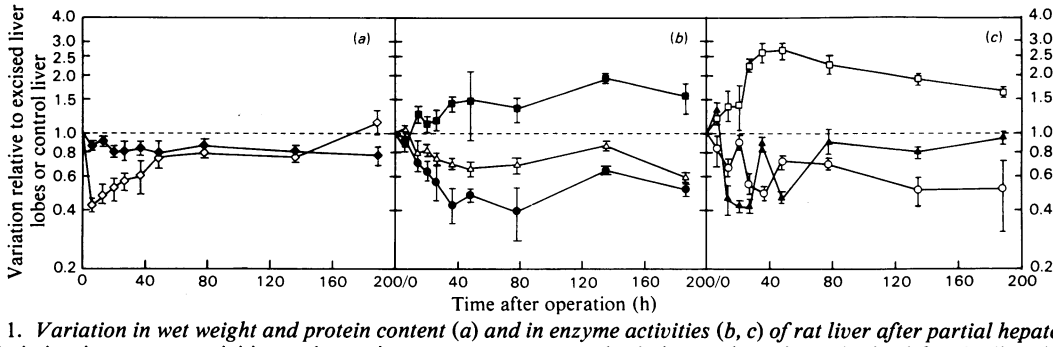

Fig. 1. *Variation in wet weight and protein content (a) and in enzyme activities (b, c) of rat liver after partial hepatectomy*
Variation in enzyme activities and protein content are expressed relative to the values obtained for the liver lobes excised at zero time from the animals ($n = 29$). Variation in liver wet weight is expressed relative to the wet liver weight of non-operated control animals ($n = 3$). Means ± S.D. for three animals are given. (*a*): ◇, wet liver weight; ♦, protein content; zero-time values were 7.65 ± 1g and 262 ± 4mg/g of wet liver respectively. (*b*): ■, Sialyltransferase; ●, CMP-*N*-acetylneuraminate hydrolase; △, synthetase; zero-time values were 0.0188 ± 0.0007, 3.02 ± 0.05 and 0.378 ± 0.008μmol/min per g of wet liver respectively. (*c*): □, Galactosyltransferase; ○, UDP-galactose pyrophosphatase; ▲, phosphodiesterase I; zero-time values were 0.0040 ± 0.0001, 33.5 ± 0.6 and 34.7 ± 0.7μmol/min per g of wet liver respectively.

decreased markedly shortly after partial hepatectomy (Figs. 2 and 3). Restoration to control values appeared to take place within the first 40–60 h after partial hepatectomy.

The increased glycosylation capacity of the regenerating liver is paralleled by decreased activities of the nucleotide-sugar-hydrolysing enzymes CMP-*N*-acetylneuraminate hydrolase (Fig. 1*b*), UDP-galactose pyrophosphatase and phosphodiesterase I (Fig. 1*c*), a non-specific pyrophosphatase/phosphodiesterase (Bischoff *et al.*, 1976; Blomberg & Berzins, 1980). In sham-operated animals the same phenomenon was noticed, although to a minor extent (results not shown). So for the second time a possible relationship between nucleotide-sugar-hydrolysing enzymes and glycosylation was observed; Nelson *et al.* (1977) reported the same phenomenon for UDP-galactose pyrophosphatase activity in the endometrium of rats during the oestrous cycle.

Slightly elevated concentrations of nucleotide sugars have been reported for regenerating rat liver (Bauer *et al.*, 1976; Okamoto & Akamatsu, 1977) and are suggested from our results for CMP-*N*-acetylneuraminate (Fig. 4). For CMP-*N*-acetylneuraminate, synthesis *de novo* is unlikely to account for this increase, since the liver CMP-*N*-acetylneuraminate synthetase activity is lowered

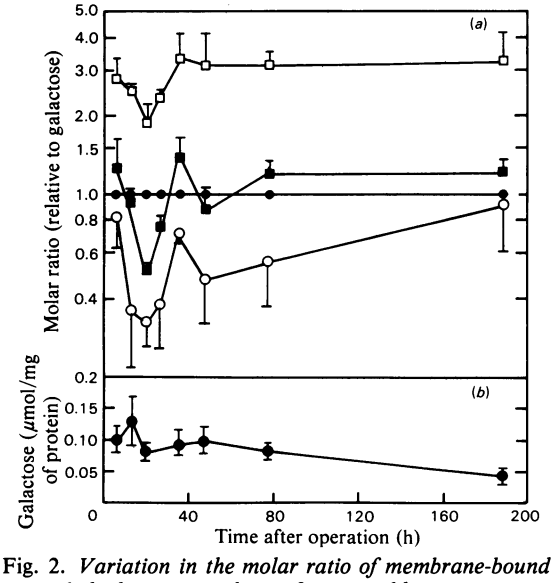

Fig. 2. *Variation in the molar ratio of membrane-bound carbohydrates in rat liver after partial hepatectomy*
(*a*) Molar ratio of mannose (□), *N*-acetylglucosamine (■) and *N*-acetylneuraminate (○) relative to galactose (●). (*b*) Variation of galactose in μmol/mg of membrane protein. Values are means ± S.D. for three animals.

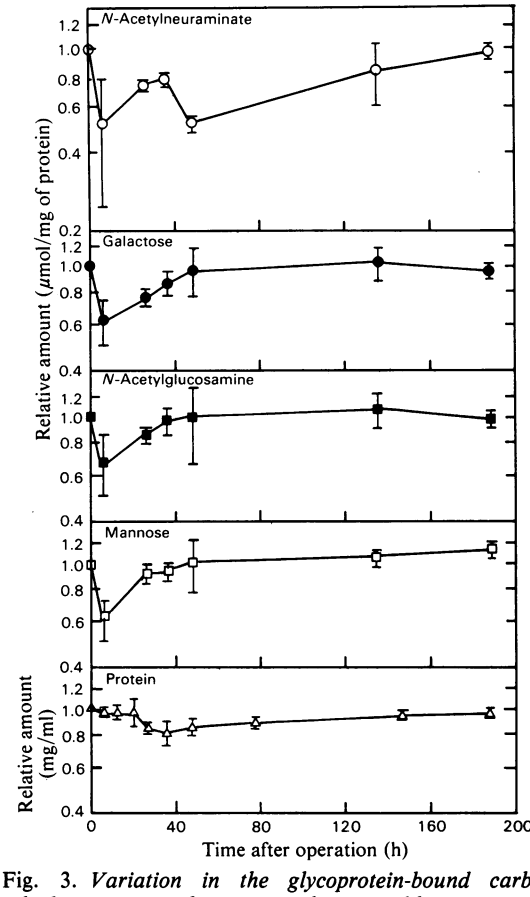

Fig. 3. *Variation in the glycoprotein-bound carbohydrate content of rat serum after partial hepatectomy*
Values ± S.D. ($n=3$) are expressed relative to the values obtained in control rat serum for carbohydrates (μmol/mg of serum protein) and serum protein (mg/ml).

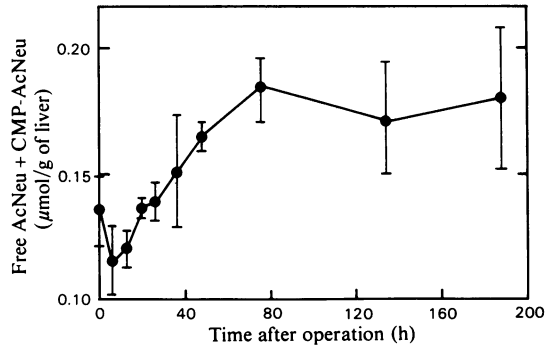

Fig. 4. *Variation in the amount of free N-acetylneuraminate (AcNeu) + CMP-N-acetylneuraminate (CMP-AcNeu) in rat liver after partial hepatectomy*
Means ± S.D. for three rat livers are given.

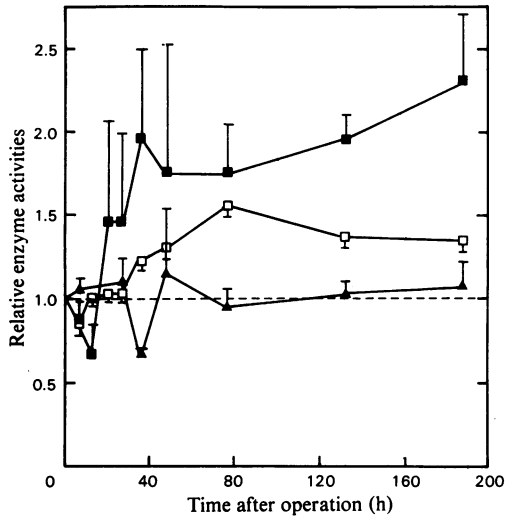

Fig. 5. *Variation in the activities of sialyltransferase* (■), *galactosyltransferase* (□) *and phosphodiesterase I* (▲) *in rat serum after partial hepatectomy*

The enzyme activities are expressed relative to the activities in serum of control rats [$(1.71 \pm 0.08) \times 10^{-4}$, $(3.00 \pm 0.07) \times 10^{-4}$ and 0.37 ± 0.01 μmol/min per ml of serum respectively]. Means ± S.D. for three animals are given.

after partial hepatectomy (Fig. 1*b*). The elevated concentrations of nucleotide sugars might therefore result from decreased activities of nucleotide-sugar-hydrolysing enzymes.

The decreased activities of the nucleotide-sugar-hydrolysing enzymes most probably are not due to increased secretion of the enzymes into blood, since the very low serum activities of these enzymes were not affected significantly after operation, as is shown for phosphodiesterase I in Fig. 5. Increased secretion might be responsible for the elevation of the serum glycosyltransferase activities after operation (Fig. 5), since these elevations paralleled the increases in the glycosyltransferase activities in liver (see also Ip, 1979). The different activity profiles presented in Fig. 1 for the various nucleotide-sugar-hydrolysing enzymes do confirm the suggestion (Van Dijk *et al.*, 1977) that, in liver, besides a non-specific nucleotide-sugar pyrophosphatase (phosphodiesterase I) there also exist other hydrolysing enzymes, namely CMP-*N*-acetylneuraminate hydrolase and UDP-galactose pyrophosphatase.

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