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Activity of glycogen synthase and glycogen phosphorylase in normal and cirrhotic rat liver during glycogen synthesis from glucose or fructose



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ABSTRACT

Cirrhotic patients often demonstrate glucose intolerance, one of the possible causes being a decreased glycogen-synthesizing capacity of the liver. At the same time, information about the rates of glycogen synthesis in the cirrhotic liver is scanty and contradictory. We studied the dynamics of glycogen accumulation and the activity of glycogen synthase (GS) and glycogen phosphorylase (GP) in the course of 120 min after per os administration of glucose or fructose to fasted rats with CCl₄-cirrhosis or fasted normal rats. Blood serum and liver pieces were sampled for examinations. In the normal rat liver administration of glucose/fructose initiated a fast accumulation of glycogen, while in the cirrhotic liver glycogen was accumulated with a 20 min delay and at a lower rate. In the normal liver GS activity rose sharply and GP activity dropped in the beginning of glycogen synthesis, but 60 min later a high synthesis rate was sustained at the background of a high GS and GP activity. Contrariwise, in the cirrhotic liver glycogen was accumulated at the background of a decreased GS activity and a low GP activity. Refeeding with fructose resulted in a faster increase in the GS activity in both the normal and the cirrhotic liver than refeeding with glucose. To conclude, the rate of glycogen synthesis in the cirrhotic liver is lower than in the normal one, the difference being probably associated with a low GS activity.

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1. Introduction

Chronic hepatitis is a common human pathology. Its final and the most life-threatening stage is liver cirrhosis, which results in considerable reconstructions of the liver's structure and the changes of its metabolism (Bircher et al., 1999). Some characteristic features of this altered metabolism are: (1) energy is produced by oxidation of lipids rather than carbohydrates; (2) following nocturnal fasting, glucose is produced by gluconeogenesis rather than by glycogenolysis; (3) ketogenesis is enhanced (Petersen et al., 1999; Bechmann et al., 2012). Carbohydrate metabolism during liver cirrhosis is somewhat similar to that during diabetes, a disease primarily characterized by glucose and insulin intolerance (Schneiter et al., 1999; Kawaguchi et al., 2011). One of the causes of glucose intolerance in

cirrhotic patients may be a decreased glycogen-synthesizing capacity of the liver.

Carbohydrates are the major component of the human diet. The most common dietary sugars are glucose and fructose, contained in the food both in the free form and as part of oligosaccharides, polysaccharides, glycosides and other derivatives. Contrary to glucose, absorption of fructose by the liver is not insulin-dependent. Therefore, fructose infusions to cirrhotic patients may not have the side effects associated with the infusion of glucose (Elliott et al., 2002). Fructose is also considered to bring about a faster accumulation of glycogen in the liver than glucose (Niewoehner et al., 1984a; Gitzelmann et al., 1989).

Glycogen accumulation in the cirrhotic liver is poorly studied. Information on its rates and the activity of the involved enzymes, glycogen synthase (GS) and glycogen phosphorylase (GP), is contradictory. Inconsistency of the results reported by different authors is mostly due to the fact that they were obtained from heterogeneous material (with various degrees of liver pathology expression and at various stages of the digestive cycle).

In this paper we report on the dynamics of glycogen accumulation and the activity of GS and GP in the normal and the cirrhotic

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liver of fasted rats at different stages after per os administration of glucose or fructose.

2. Materials and methods

2.1. Animals

We used 102 outbred male white rats weighing 130–140 g in the beginning and 250–300 g in the end of the experiment. The rats were given a standard diet. In the beginning of the experiment the animals were separated into two groups, the experimental one and the control one. Liver cirrhosis in rats from the experimental group was produced by chronic inhalation of CCl₄ vapours (7 ml per 100 l of the closed chamber volume) in the course of six months; the animals were treated three times weekly for 20 min. Rats from the control group were untreated.

A week after the termination of treatment with CCl₄, 5 rats from the control and 5 rats from the experimental group were decapitated after nocturnal fasting. Their blood serum samples and pieces of liver were used for histological and biochemical research. To estimate the dynamics of glycogen accumulation in the liver, the rats were fasted for 48 h (water ad libitum) and then administered per os 30% solution of glucose or fructose (4 g per 1 kg of body weight). The rats were decapitated immediately after the termination of fasting and 10, 20, 30, 45, 60, 75, 90 and 120 min after the administration of monosaccharides.

The experiments described here were performed in accordance with the “Guide for the care and use of laboratory animals” (<http://www.nap.edu/catalog/10498.html>) and “On the statement of rules of laboratory practice” (http://www.zdrav.spb.ru/official_documents/MZ/list_mz2003.htm).

2.2. Histological methods

Pieces of rat liver were fixed in 10% neutral formaldehyde and embedded in paraffin blocks, which were cut with the Reichert microtome (Austria) into sections ca. 5 µm thick. The tissue sections were stained with haematoxylin–eosin (Biovitrum, Russia) after Romanovsky and picro-fuchsin after Van Gieson (Pearce, 1962). The stained sections were microscopically analyzed with the help of the Axiovert 200 M microscope (Carl Zeiss, Germany) equipped with 10 × 0.30 and 40 × 0.75 objectives. The relative volume of the connective tissue and the parenchyma of the rat liver in the sections was determined after (Weibel et al., 1969) using an eyepiece reticule (16 × 16), a 25 × 0.50 objective and an 8 × eyepiece.

2.3. Biochemical study of the liver

The concentration of total bilirubin (TB), total protein and the activity of alanine aminotransferase (ALT), aspartate aminotransferase (AST) in the rat blood serum was determined with the help of the Abbot-spectrum automatic biochemical analyzer (Abbott Laboratories S.A., USA).

2.4. Determination of the activity of antioxidant enzymes and the concentration of lipid peroxidation (LP) products in the liver

For this, we used 10% tissue homogenates made on 25 mM Tris–HCl buffer with 175 mM KCl (pH 7.4). The activity of superoxide dismutase (SOD) was estimated by the reduction of nitro blue tetrazolium with NADH and phenazine methosulphate (Nishikimi et al., 1972). Catalase activity was assessed by the rate of hydrogen peroxide decomposition (Luck and Catalase, 1971). The concentration of LP products, diene conjugates (DC) and malone dialdehyde

(MDA) was determined, respectively, after Romanova and Stalnaya (1977) and after Uchigama and Michera (1978).

2.5. Determination of glucose concentration in the blood

Glucose concentration in the blood serum of rats was determined by an enzyme colorimetric assay using the standard kit (Olvex Diagnosticum, Russia). The assay is based on the glucose oxidation reaction in the presence of glucose oxidase with the formation of hydrogen peroxide, which, in turn, oxidizes o-toluidine in the presence of peroxidase with the formation of stained products. After adding perchlorate, experimental samples were centrifuged at 900 × g for 10 min. Working reagent was added to the obtained supernatant in the proportion of 1:200; after stirring, the mixture was incubated at room temperature for 30 min. Experimental and calibration samples were measured against the control sample at 500 nm using the Specol 11 spectrophotometer (Carl Zeiss, Germany) fitted with 1 cm cells.

2.6. Determination of glycogen content

Glycogen content was determined in the sediment formed after adding 1.2 volumes of ethanol to the lysate of liver tissues (Vilkova, 1982). Liver tissues were lysed in 30% KOH in a boiling water bath for 60 min. After centrifuging for 30 min at 1000 × g, the glycogen pellet was washed in an ascending ethanol series (70, 80 and 96%) and then centrifuged again. The pellet obtained after the centrifuging was hydrolysed in 2 N H₂SO₄ in a boiling water bath for 2.5 h. The hydrolysate was neutralized with 5 N NaOH up to pH 7.8–8.0 and the amount of the glucose formed was determined by glucose oxidase method using the standard kit.

2.7. Determination of GS and GP activity

Rat liver was homogenized in 50 mM Tris–HCl buffer (pH 7.4) with 5 mM ethylenediaminetetraacetic acid (EDTA), 200 mM sucrose, 0.01 M β-mercaptoethanol and 0.2 M phenylmethylsulfonyl fluoride on ice (1:10). The homogenate was centrifuged at 1000 × g and 4 °C for 10 min to remove incompletely destroyed cells and nuclei. The supernatant was then centrifuged at 14,000 × g and 4 °C for 10 min. The newly obtained supernatant was used to determine the activity of GS and GP by the substrate-labelled assay.

GS activity was estimated by the amount of [U-¹⁴C]glucose included in the glycogen, using UDP-[U-¹⁴C]glucose (300 mCi/mM) as a substrate (Vardanis, 1992). To determine the activity of the enzyme, 40 µl of the sample was added to 75 µl of 10 mM Tris–HCl buffer (pH 7.5) containing 0.17 mM EDTA, 100 mM NaF, 2.5 mM MgSO₄, 10% glycogen and 0.25 mM UDP-glucose (40,000 imp/min). The mixture was incubated for 10 min at 30 °C in the presence of 7.2 mM glucose-6-phosphate during determination of the activity of the enzyme's D-form and in the presence of 0.17 mM glucose-6-phosphate during determination of the activity of the enzyme's I-form. The reaction was arrested by application of 0.5 ml of 96% ethanol onto 1.5 cm² filters (Whatman 3 mm) with 50 µl of the reactive mixture. After the reaction arrest, the filters were rinsed twice (30 min each time) in 66% ethanol and once in acetone for 5 min in order to remove the non-included label. To count the impulses, dried filters were placed into vials with a liquid scintillator ZHS-7. The number of impulses was registered with a counter (Beckman, USA).

GP activity was determined using the inverse reaction of glycogen synthesis in vitro. The amount of [U-¹⁴C]glucose incorporated into glycogen was measured using [U-¹⁴C]glucose-1-phosphate (286 mCi/mM) as a substrate (Vardanis, 1992). The reaction was performed in 50 mM Tris–HCl buffer (pH 6.7) containing 100 mM NaF, 10% glycogen, 10 mM glucose-1-phosphate (35,000 imp/min).

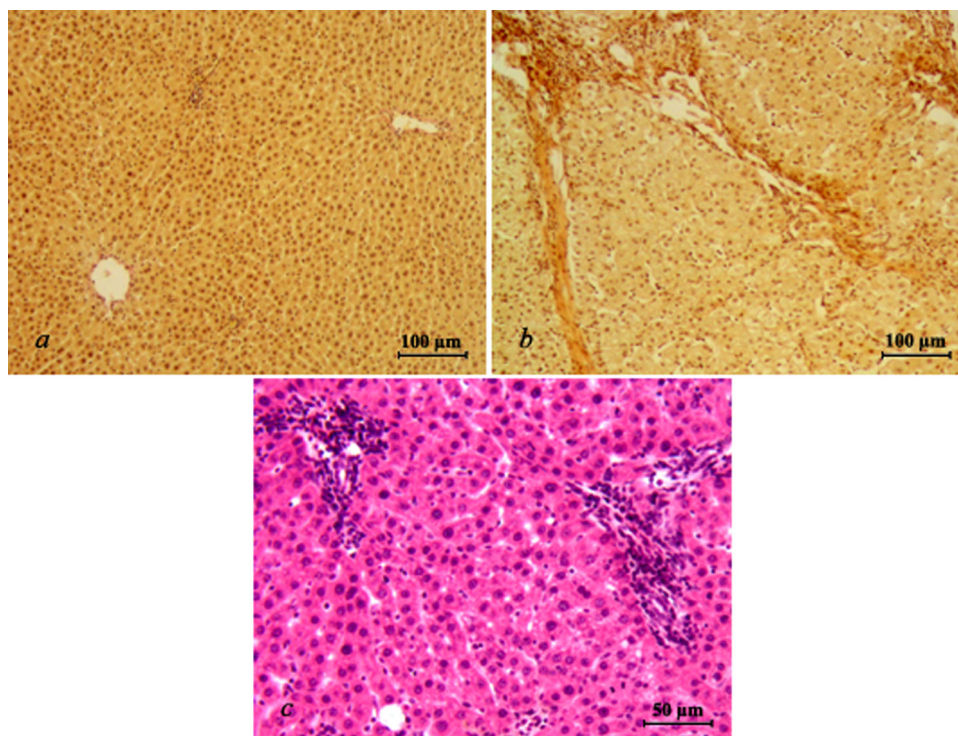


Fig. 1. Sections of liver from a normal rat (1) and rats with liver cirrhosis induced by chronic poisoning with carbon tetrachloride (2, 3). Sections were stained with van Gieson (1, 2) or haematoxylin–eosin stains (3). Objective 10 \times /0.30 (1, 2) and objective 20 \times /0.50 (3).

During determination of the α -form of the enzyme, 0.5 mM of caffeine was added to the reactive mixture; β -form of GP was activated by addition of 10 mM of AMP. The mixture was incubated at 37 °C for 5 min after the addition of 35 μ l of the sample. Impulses were then counted as described above.

The enzyme activity depended on the protein content in the sample and was proportional to the incubation time. The protein content in the sample was determined according to Bradford (1976), with Coomassie brilliant blue G-250 as the dye. Protein content was read from the standard calibration curve constructed from measurements of various amounts of bovine serum albumin made at 595 nm with the use of the Specol 11 spectrophotometer (Carl Zeiss, Germany).

Statistical treatment of the results was performed using Sigma Plot for Windows 11.0 standard software package (Systat Software Inc., Chicago, IL, USA). The data were given as mean \pm standard error of the mean. Differences between the mean values were detected using Student's t -criterion. Fisher's exact test (F -test) was used to analyze the dynamics of glucose concentration in the blood of the normal and the cirrhotic animals. Three-factor dispersion analysis (ANOVA) was used to evaluate the relationships between glycogen concentration, GS activity, GP α activity and time of refeeding, the type of sugar and various rat group.

3. Results

Rats treated with CCl₄ for 6 months developed a typical liver cirrhosis characterized by the distortion of the normal lobular structure and the presence of leukocytic infiltration foci (Fig. 1). Morphometric analysis of the normal and the cirrhotic liver showed that in the latter the proportion of parenchyma decreased by 5% and that of connective tissue increased by 317% ($p < 0.001$). As a result, the parenchyma occupied $88.0 \pm 1.1\%$ in the normal tissue as compared to $83.0 \pm 1.5\%$ in the cirrhotic one ($p < 0.05$).

Liver cirrhosis in rats was accompanied by an increase in the levels of ALT and AST by 94.6% and 42.8%, correspondingly, as well as by a 1.7-fold increase in the TB concentration. At the same time, total protein concentration in the blood of rats from the experimental group was 22% lower than in the control (Table 1).

As shown in Table 2, the rate of SOD activity in the cirrhotic rats decreased by 16% and that of catalyze activity, by 23%. DC concentration in the cirrhotic liver was increased by 107% as compared to the norm, while the level of MDA increased by 52%.

Glucose concentration in the blood of animals from the control and the experimental group after 48 h of fasting was the same. Administration of glucose or fructose resulted in a rapid increase in glucose concentration in the blood of rats from both groups. However, at almost all times after the start of refeeding the concentration of glucose in the blood of cirrhotic rats was lower than in the control (Fig. 2). Comparison of the glucose dynamics in the blood of the control and the experimental rats (Fisher's test) showed that after administration of both glucose ($F = 5.11$, $p < 0.05$) and fructose ($F = 4.63$, $p < 0.05$) the average level of these processes in the normal rats was higher than in the cirrhotic ones.

Administration of glucose or fructose stimulated the synthesis of glycogen in the liver. In the normal liver the glycogen content was almost twice higher ($p < 0.01$) in fed rats than in fasted rats as early as 10 min after feeding, whereas in the cirrhotic liver a noticeable increase in the glycogen content was observed only 20 min after the administration of sugar. After that the glycogen content in the liver of rats from both groups continued to increase; however, at all times except 120 min after administration of monosaccharides the level of glycogen in the normal liver was higher than in the cirrhotic one (Fig. 3). Three-factor dispersion analysis showed that glycogen concentration in the liver was influenced by: (a) time since refeeding, (b) type of sugar (glucose/fructose), (c) type of animals (control/experimental) ($p < 0.001$).

The activity of GS after 48 h fasting in the normal and the cirrhotic liver was at the same low level. Administration of

Table 1
Biochemical factors of blood serum in normal and cirrhotic rats.

Rats	ALT activity, U/L	AST activity, U/L	Total bilirubin, $\mu\text{mol/L}$	Total protein, g/L
Normal	143.2 \pm 5.2	160.8 \pm 8.1	0.34 \pm 0.04	6.46 \pm 0.14
Cirrhotic	278.7 \pm 9.1 ^c	228.0 \pm 14.6 ^b	0.58 \pm 0.04 ^b	5.04 \pm 0.03 ^a

Values are means with their standard errors for five rats.

^a Different from the control with the following significance level: $p < 0.05$.

^b Different from the control with the following significance level: $p < 0.01$.

^c Different from the control with the following significance level: $p < 0.001$.

Table 2
Some indices of microsomal oxidation and lipid peroxidation in the liver of normal and cirrhotic rats.

Rats	MDA, $\mu\text{mol/g}$	SOD, E/mg of protein	Catalase, mmol $\text{H}_2\text{O}_2/\text{min}$	DC, $\mu\text{mol/g}$
Normal	20.52 \pm 1.56	4.00 \pm 0.17	95.78 \pm 2.48	55.84 \pm 0.94
Cirrhotic	31.14 \pm 2.69 ^a	3.36 \pm 0.16 ^a	74.12 \pm 1.96 ^a	115.74 \pm 4.67 ^b

Values are means with their standard errors for five rats.

^a Different from the control with the following significance level: $p < 0.05$.

^b Different from the control with the following significance level: $p < 0.001$.

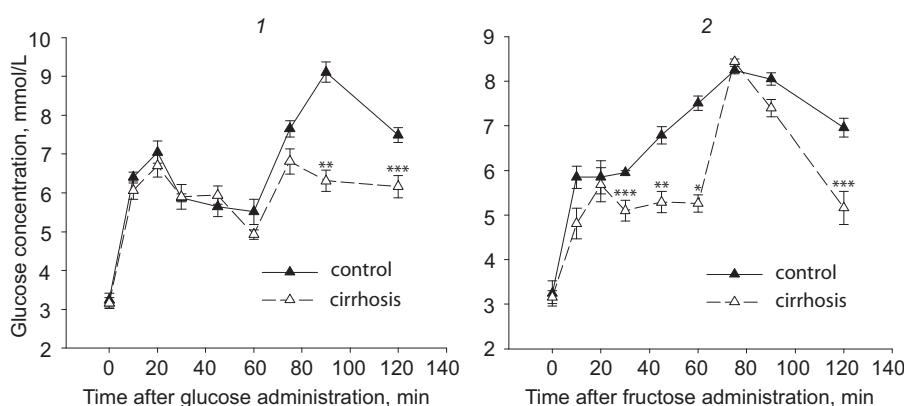


Fig. 2. Glucose concentration in the blood serum of the control and the cirrhotic rats after administration of glucose (1) or fructose (2) to fasted animals. Abscissa – glucose concentration, mmol/L; ordinate – time after glucose or fructose administration, min. $n = 3$, for each time point. * $p < 0.001$; ** $p < 0.01$; *** $p < 0.05$ – mean values were significantly different from control.

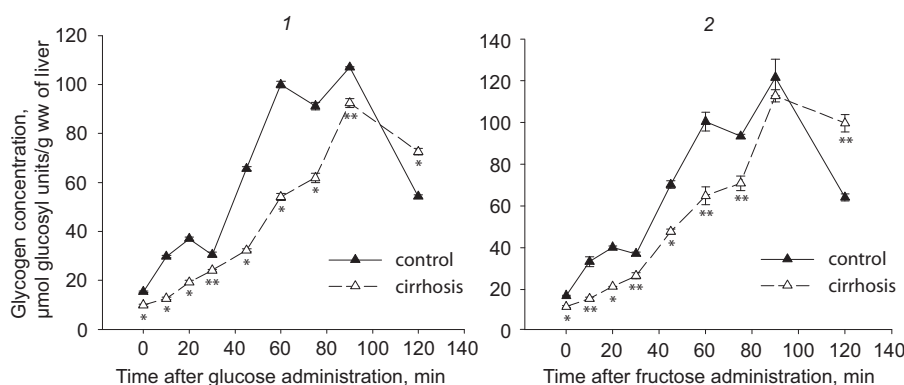


Fig. 3. Dynamics of glycogen accumulation in the normal (control) and the cirrhotic rat liver after administration of glucose (1) or fructose (2) to fasted animals. Abscissa – glycogen concentration, $\mu\text{mol glucosyl units/g (ww)}$ of liver; ordinate – time after glucose or fructose administration, min. $n = 3$, for each time point. * $p < 0.001$; ** $p < 0.01$ – mean values were significantly different from control.

glucose to fasted rats resulted in a rapid increase in the activity of this enzyme. After 20 min GS activity in the liver of rats from the control and the experimental group increased as compared to that in the liver of fasted rats by 263% and 235%, correspondingly. Administration of fructose resulted in a sharper increase in the GS activity as compared to administration of glucose, both in the normal and the cirrhotic liver. Twenty minutes after fructose administration GS activity in the liver of rats from the control and the experimental group increased by 358% and 326%,

correspondingly. After that, in the interval of 20–75 min following the administration of glucose, GS activity continued to grow, whereas after the administration of fructose it remained at approximately the same level. The activity of the enzyme in the cirrhotic liver, having reached its maximum at 75 min after the start of refeeding, did not change until the end of the experiment, whereas in the normal liver it decreased after 75 min (Fig. 4). Nevertheless, in the interval of 0–90 min the activity of GS in the cirrhotic liver after administration of monosaccharides was, on the average, by 6.2%

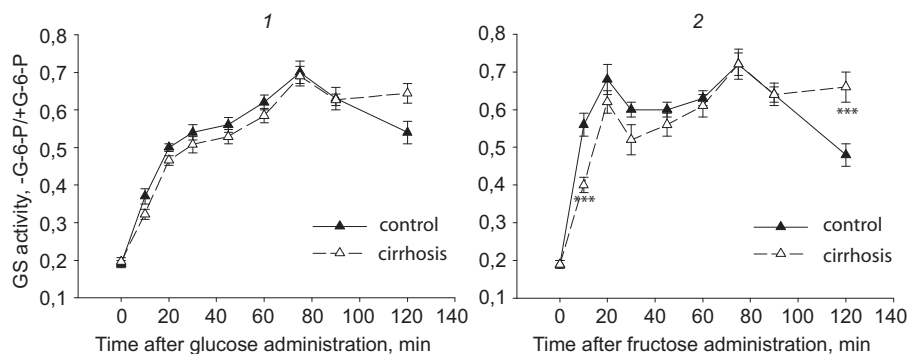


Fig. 4. Activity of glycogen synthase (GS) in the normal (control) and the cirrhotic rat liver after administration of glucose (1) or fructose (2) to fasted animals. Abscissa – GS activity, -G-6-P/+G-6-P, ordinate – time after glucose or fructose administration, min. $n=3$, for each time point. $***p < 0.05$ – mean values were significantly different from control.

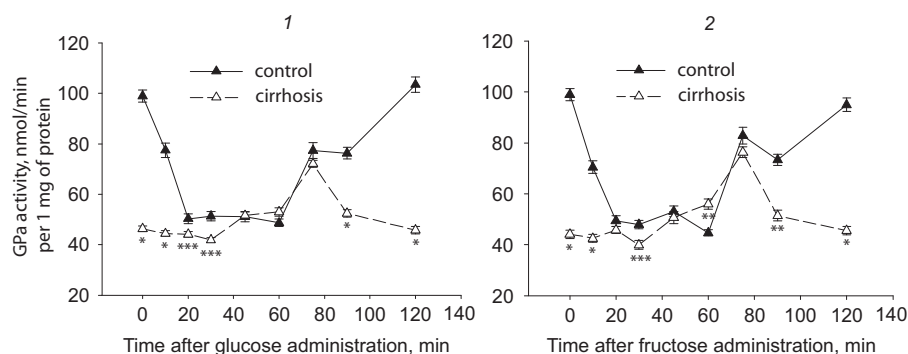


Fig. 5. Activity of glycogen phosphorylase α (GP α) in the normal (control) and the cirrhotic rat liver after administration of glucose (1) or fructose (2) to fasted animals. Abscissa – GP α activity, nmol/min per 1 mg of protein, ordinate – time after glucose or fructose administration, min. $n=3$, for each time point. $*p < 0.001$; $**p < 0.01$; $***p < 0.05$ – mean values were significantly different from control.

lower than in the normal liver ($p < 0.01$). ANOVA showed that glycogen synthase activity in the liver was influenced by: (a) time since refeeding ($p < 0.001$), (b) type of sugar (glucose/fructose) ($p < 0.001$), and (c) type of animals (control/experimental) ($p < 0.01$).

The activity of the α -form of GP (Fig. 5) after 48 h of fasting, though high in the liver of animals from both groups, was by 55% ($p < 0.001$) higher in the normal liver than in the cirrhotic one. Administration of both glucose and fructose brought about a rapid decrease in GP α activity in the normal liver. As a result, already 20 min after the start of the refeeding the enzyme activity was decreased twice as compared to fasted rats. GP α activity remained the same for the next 40 min and began to grow after that, reaching the levels characteristic of the normal liver of fasted rats 120 min after the administration of glucose or fructose.

Contrary to the liver of the control animals, GP α activity in the cirrhotic liver during the first 30 min after the start of the refeeding remained at the same low level as in fasted rats. After that the activity of this enzyme increased gradually, reaching the maximum 75 min after the start of refeeding with glucose or fructose. This maximum, though being 56–73% higher than the level of GP α activity in the liver of fasted rats, was by 25–30% lower than the maximum activity of GP α in the normal liver. After that, the activity of the enzyme in the cirrhotic liver, contrary to the normal one, decreased, by 120 min reaching the level observed before the start of the refeeding. On the whole, the activity of GP α in the liver of rats from the experimental group was by 26% lower than in the normal liver of the control rats ($p < 0.01$).

Three-factor dispersion analysis showed that GP α activity was influenced by the time since refeeding ($p < 0.001$) and the type of animals (control/experimental) ($p < 0.001$) but not the type of sugar ($p > 0.05$).

4. Discussion

After 6 months of treatment with CCl₄ rats developed liver cirrhosis, morphologically similar to that in humans (Planaguma et al., 2005). Pathological changes in the liver are thought to be triggered by excessively intense LP reactions. The increased concentrations of LP products and the damage to the membrane structures of the cell are brought about by the decreased activity of the antioxidant enzymes in the liver (Ohyashiki et al., 1995). In the liver of cirrhotic rats the activity of the key antioxidant enzymes, catalase and SOD, was decreased, whereas the concentration of LP products, MDA and DC, was increased (Table 2). Thus, our results confirm the findings of other authors (Huang et al., 2003; Zhan et al., 2011). LP-induced destruction of membranous structures of hepatocytes results in cytolysis, revealed by the increased ALT and AST levels in the blood serum (Table 1), indicating continuous death of hepatocytes in the pathologically changed liver.

In our study the fraction of parenchyma in cirrhotic rat liver was decreased, whereas the fraction of connective tissue was increased. A similar dynamics of the connective tissue and parenchyma in the cirrhotic liver was observed by other authors (James et al., 1986; Reichen et al., 1987; Gressner and Schuppan, 1999). Increased concentrations of TB and decreased total protein in cirrhotic rats (Table 1) imply a disturbance of pigment metabolism and protein synthetic function in the liver. Similar results were obtained in many studies (Harvey et al., 1999; Zhan et al., 2011).

Considerable reconstructions of the cirrhotic liver, accompanied by hepatocellular deficiency, hypoxia and impaired cell metabolism, suggest the impairment of the glucostatic function of the liver. Based on this, one might have expected the glucose concentration in the blood of fasted cirrhotic rats or humans to be lower than in fasted normal ones. However, most of the literature

data appear to indicate that it falls within the range of the norm (Johansson et al., 1994; Riggio et al., 1997; Petersen et al., 1999). In our study, too, glucose levels in the blood of experimental rats after 48 h of fasting were the same as in the normal ones (Fig. 2).

Increased concentration of glucose in the blood after its administration to fasted rats resulted in a rapid accumulation of glycogen in the normal liver. The rate of glycogen synthesis after per os administration of glucose to fasted rats varied from 0.56 to 1.7 $\mu\text{mol}/\text{min}$ (Niewoehner et al., 1984b; Kuwajima et al., 1986). In our study, in the interval of 0–30 min, the former made up 2.3 $\mu\text{mol}/\text{min}$ per 1 g of the liver.

Information about glycogen synthesis in the cirrhotic liver is scanty and contradictory. On the one hand, it was shown that the rates of glycogen synthesis from glucose in the liver of cirrhotic patients after nocturnal fasting did not differ from that in the control (Kruszynska et al., 1993; Schreiber et al., 1999). On the other hand, it was found that glycogen content in the cirrhotic liver of humans and rats after feeding was much lower than in the normal one (Giardina et al., 1994; Riggio et al., 1997; Krähenbühl et al., 2003). In our study, on the whole, the rates of glycogen accumulation in cirrhotic rats were lower than in the norm (Fig. 3).

The decreased rates of glycogen accumulation in the cirrhotic liver may be explained both by a slackening of glycogenesis and an enhancement of glycogenolysis. As known, GS and GP play a key role in these processes. Administration of glucose to the rats from the control group stimulated a rapid increase in the GS activity (Fig. 4a), reported in many other studies (Niewoehner et al., 1984b; Niewoehner and Nuttall, 1988; Ercan et al., 1994). As compared to glucose, fructose brought about an even faster increase in the GS activity, with its maximum reached earlier than after the administration of glucose (Fig. 4b). Other authors, however, failed to observe any differences in the levels of GS activity in the normal liver after the refeeding of rats with glucose or fructose (Ciudad et al., 1980; Niewoehner et al., 1984a).

Contrary to the normal liver, there is no information about the dynamics of GS activity in the cirrhotic liver in the course of refeeding with glucose or fructose. There are only contradictory records of the activity of this enzyme at some, often uncertain, time after the start of refeeding (Giardina et al., 1994; Krähenbühl et al., 1996). Most of the available data indicate that in the post-absorption period GS activity in the cirrhotic liver does not differ much from the norm (Krähenbühl et al., 1991; Vardanis, 1992; Kudryavtseva et al., 1998, 1999). In our study, too, the activity of GS in the cirrhotic liver after the administration of glucose or fructose was only slightly lower than in the liver of rats from the control group (Fig. 4). Since the activity of GP is much higher than that of GS, the former plays the leading role in the control of glycogen metabolism in the liver (Roach, 2002; Ferrer et al., 2003). GP_a, the active form of GP, is a strong inhibitor of phosphatase synthase, and so the decrease in its activity allows the activation of GS and the start of glycogen synthesis in the cells. Based on this, it was thought that GP_a inactivation by its transformation into the dephosphorylated *b*-form is an indispensable condition for the initiation of glycogen synthesis in the liver (Hers, 1990). In our study, though the GS activity was maintained at the same high level, the activity of GP_a grew to reach the initial level by 120 min (Fig. 5). Reciprocal interaction of GS and GP_a during glycogen synthesis was demonstrated in numerous studies (Minassian et al., 1999; Aiston et al., 2003). A simultaneously high activity of GS and GP_a in the normal liver, when the glycogen content in hepatocytes increases considerably, indicates that synthesis and breakdown of glycogen occur simultaneously. Thus, an increase in the glycogen concentration in the liver may be an important factor stimulating glycogen turnover and preventing its uncontrolled accumulation. The presence of glycogen turnover is supported by the direct dependence between the rate of glycogenolysis and the concentration of glycogen in the liver (Magnusson et al., 1994).

Information on the GP activity in the cirrhotic liver is scanty and uncertain. Krähenbühl and colleagues did not find any differences between the levels of the total and the active GP forms in the liver of the CCl₄-cirrhotic rats and those in the normal liver. However, the same authors recorded the decrease in both total and active GP in the liver of rats with biliary cirrhosis (Krähenbühl et al., 1991, 1996). In our study the initiation of glycogen synthesis and its further accumulation occurred at the background of a weak GP_a activity, with the exception of a short-term increase after 75 min (Fig. 5). These results agree well with those from our earlier study, where we showed that the GP_a activity in the liver of cirrhotic rats dropped by 15–35% below the norm (Kudryavtseva et al., 1999, 2003). In our experiments cirrhotic rats demonstrated a decrease in the levels of both active and total GP (by 29%, $p < 0.001$), which might indicate a slackening of the synthesis of this enzyme.

Regulation of GS and GP activity in the cirrhotic liver is poorly studied. The activity of these enzymes is known to be determined by multiple factors. The degree of GS activation directly depends on the intracellular concentration of glucose-6-phosphate (G-6-P) (Roach, 2002). G-6-P is also known to be an allosteric inhibitor of GP (Biorn and Graves, 2001). In the normal liver G-6-P concentration, low in fasted rats, increases rapidly after per os administration of glucose or fructose, resulting in the activation of GS and the inhibition of GP_a. However, an increase in the GP_a activity and the maintenance of the high GS activity 60 min after the start of refeeding cannot be explained only by the dynamics of G-6-P, since the concentration of the latter decreases after the administration of glucose, remaining at approximately the same levels for 2 h after the administration of fructose (Niewoehner et al., 1984a,b).

G-6-P concentration in the cirrhotic liver of fasted rats and its further dynamics in the course of refeeding with glucose or fructose are unknown. We may venture a guess that G-6-P concentration in cirrhotic liver may exceed the norm due to the high rate of gluconeogenesis, as well as reduce the activity of glucose-6-phosphatase and hepatic glucose production (Shimamura, 1987; Kruszyńska et al., 1999; Kudryavtseva et al., 1999, 2001). Finally, as shown in numerous studies, glucose production in the cirrhotic liver is lower than in the normal one (Merli et al., 1986; Johansson et al., 1994). As a result, high G-6-P concentrations may suppress the activity of GP.

Low levels of GP_a in the cirrhotic liver can be caused by several other factors. To begin with, cAMP is known to be an important modulator of the GP activity, the increase in its concentration in cells resulting in an enhanced activity of protein kinase and in the GP_a activation. The levels of adenylate cyclase, and its product cAMP, in the cirrhotic liver are much lower as compared to the norm (Zhao et al., 2011). The concentration of B₆ vitamin also influences considerably the GP activity, altering the conformation of this enzyme. During B₆-avitaminosis GP activity decreases greatly, whereas that of GS remains unchanged (Rosenfeld and Popova, 1989). B₆ content in the cirrhotic liver of humans is 2–3 times lower than in the normal one (Ohgi and Hirayama, 1988; Henderson et al., 1989).

To sum up, glycogen metabolism in the cirrhotic liver is considerably modified as compared to the norm. The changes are first of all associated with a smaller increase in the GS activity after the administration of monosaccharides and a decreased rate of glycogen synthesis at the background of a constantly low GP_a activity. Administration of fructose instead of glucose to fasted rats results in minor changes in the dynamics of glycogen content and GS activity in the normal and the cirrhotic liver.

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Table A1
Three way analysis of variance.

Source of variation	DF	SS	MS	F	p
Glucose/fructose	1	1666.949	1666.949	13.239	<0.001
Normal/cirrhotic rat liver	1	55,955.916	55,955.916	444.401	<0.001
Time	8	838,374.207	104,796.776	832.294	<0.001
Glucose/fructose × normal/cirrhotic rat liver	1	690.589	690.589	5.485	0.022
Glucose/fructose × time	8	4084.969	510.621	4.055	<0.001
Normal/cirrhotic rat liver × time	8	71,537.195	8942.149	71.018	<0.001
Glucose/fructose × normal/cirrhotic rat liver × time	8	1699.292	212.411	1.687	0.116
Residual	72	9065.753	125.913		
Total	107	983,074.870	9187.616		

Table A2
Three way analysis of variance.

Source of variation	DF	SS	MS	F	p
Glucose/fructose	1	0.0850	0.0850	41.355	<0.001
Normal/cirrhotic rat liver	1	0.0179	0.0179	8.703	0.004
Time	8	2.152	0.269	130.886	<0.001
Glucose/fructose × normal/cirrhotic rat liver	1	0.00127	0.00127	0.617	0.435
Glucose/fructose × time	8	0.0927	0.0116	5.640	<0.001
Normal/cirrhotic rat liver × time	8	0.103	0.0129	6.262	<0.001
Glucose/fructose × normal/cirrhotic rat liver × time	8	0.0175	0.00219	1.064	0.398
Residual	72	0.148	0.00206		
Total	107	2.618	0.0245		

Table A3
Three way analysis of variance.

Source of variation	DF	SS	MS	F	p
Glucose/fructose	1	0.685	0.685	0.0517	0.821
Normal/cirrhotic rat liver	1	11,359.053	11,359.053	858.061	<0.001
Time	8	13,579.856	1697.482	128.227	<0.001
Glucose/fructose × normal/cirrhotic rat liver	1	140.083	140.083	10.582	0.002
Glucose/fructose × time	8	225.182	28.148	2.126	0.044
Normal/cirrhotic rat liver × time	8	12,159.740	1519.967	114.818	<0.001
Glucose/fructose × normal/cirrhotic rat liver × time	8	89.850	11.231	0.848	0.564
Residual	72	953.140	13.238		
Total	107	38,507.590	359.884		

Appendix A. ANOVA results

Dependent variable: glycogen concentration.

Normality test: failed ($p < 0.050$; Table A1).

Dependent variable: GS activity.

Normality test: failed ($p < 0.050$; Table A2).

Dependent variable: GPα activity.

Normality test: failed ($p < 0.050$; Table A3).

References

- Aiston S, Andersen B, Agius L. Glucose 6-phosphate regulates hepatic glycogenolysis through inactivation of phosphorylase. *Diabetes* 2003;52:1333–9.
- Bechmann LP, Hannivoort RA, Gerken G, Hotamisligil GS, Trauner M, Canbay A. The interaction of hepatic lipid and glucose metabolism in liver diseases. *J Hepatol* 2012;56:952–64.
- Biorn AC, Graves DJ. The amino-terminal tail of glycogen phosphorylase is a switch for controlling phosphorylase conformation, activation, and response to ligands. *Biochemistry* 2001;40:5181–9.
- Bircher J, Benhamou J-P, McIntyre N, Rizzetto M, Rodes J. Oxford textbook of clinical hepatology. Oxford: Oxford University Press; 1999.
- Bradford MM. A rapid and simple method for quantitation of microgram quantities of proteins utilizing the principle of protein-dye binding. *Anal Biochem* 1976;72:248–54.
- Ciudad CJ, Massague J, Salavert A. Synthesis of glycogen from fructose in the presence of elevated levels of glycogen phosphorylase in rat hepatocytes. *Mol Cell Biochem* 1980;30:33–8.
- Elliott SS, Keim NL, Stern JS, Teff K, Havel PJ. Fructose, weight gain, and the insulin resistance syndrome. *Am J Clin Nutr* 2002;76:911–22.
- Ercan N, Gannon MC, Nuttall FQ. Liver glycogen synthase, phosphorylase, and the glycogen concentration in rats given a glucose load orally: a 24-hour study. *Arch Biochem Biophys* 1994;315:35–40.
- Ferrer JC, Ferrer JC, Farve C, Gomis RR. Control of glycogen deposition. *FEBS Lett* 2003;546:127–32.
- Giardina MG, Matarazzo M, Sacca L. Kinetic analysis of glycogen synthase and PDC in cirrhotic rat liver and skeletal muscle. *Am J Physiol* 1994;267:E900–6.
- Gitzelmann R, Steinmann B, Van den Berghe G. Metabolic basis of inherited disease. Disorders of fructose metabolism. New York: McGraw Hill; 1989.
- Gressner AM, Schuppan D. Cellular and molecular pathobiology, pharmacological intervention and biochemical assessment of liver fibrosis. In: Bircher J, Benhamou J-P, McIntyre N, Rizzetto M, Rodes J, editors. Oxford textbook of clinical hepatology, vol. 1. Oxford: Oxford Medical Publications; 1999. p. 607–29.
- Harvey PJ, Gready GE, Hickey KM, Le Couteur DG, McLean AJ. 31P and 1H NMR spectroscopic studies of liver extracts of carbon tetrachloride-treated rats. *NMR Biomed* 1999;12:395–401.
- Henderson JM, Scott SS, Merrill AH, Hollins B, Kutner MH. Vitamin B6 repletion in cirrhosis with oral pyridoxine. *Hepatology* 1989;9:582–8.
- Hers H-G. Mechanisms of blood glucose homeostasis. *J Inher Metab Dis* 1990;13:395–410.
- Huang YT, Hsu IS, Chen CJ, Liu CT, Wie YH. Oxidative-stress-related changes in the livers of bile-duct-ligated rats. *J Biomed Sci* 2003;10:170–8.
- James J, Bosch KS, Zuyderhoudt FMZ, Houtkooper JM, Gool JV. Histophotometric estimation of volume density of collagen as an indication of fibrosis in rat liver. *Histochemistry* 1986;85:129–33.
- Johansson U, Wahren J, Eriksson LS. Splanchnic and peripheral glucose metabolism in cirrhosis. *J Hepatol* 1994;20:760–7.
- Kawaguchi T, Taniguchi E, Itou M, Sakata M, Sumie S, Sata M. Insulin resistance and chronic liver disease. *World J Hepatol* 2011;3:99–107.
- Krähenbühl L, Lang C, Ludes S, Seiler C, Shafer M, Zimmermann A, et al. Reduced hepatic glycogen stores in patients with liver cirrhosis. *Liver Int* 2003;23:101–9.
- Krähenbühl L, Talos C, Reichen J, Krahenbühl S. Progressive decrease in tissue glycogen content in rats with long-term cholestasis. *Hepatology* 1996;24:902–7.

- Krähenbühl S, Weber FL, Brass EP. Decreased hepatic glycogen content and accelerated response to starvation in rats with carbon tetrachloride-induced cirrhosis. *Hepatology* 1991;14:1189–95.
- Kruzynska YT. Carbohydrate metabolism. In: Bircher J, Benhamou J-P, McIntyre N, Rizzetto M, Rodes J, editors. *Oxford textbook of clinical hepatology*, vol. 1. Oxford: Oxford Medical Publications; 1999. p. 257–86.
- Kruzynska YT, Meyer-Alber A, Darakshan F, Home PD, McIntyre N. Metabolic handling of orally administered glucose in cirrhosis. *J Clin Invest* 1993;91:1057–66.
- Kudryavtseva MV, Bezborodkina NN, Kudryavtsev BN. Restoration of the glycogen-forming function of hepatocytes in rats with liver cirrhosis is facilitated by a high-carbohydrate diet. *Br J Nutr* 1999;81:473–80.
- Kudryavtseva MV, Bezborodkina NN, Kudryavtsev BN. Glycogen-forming function of hepatocytes in cirrhotically altered rat liver after treatment with chorionic gonadotropin. *Exp Toxicol Pathol* 2001;53:57–63.
- Kudryavtseva MV, Bezborodkina NN, Okovityi SV, Kudryavtsev BN. Effects of the 2-ethylthiobenzimidazole hydrobromide (Bemithyl) on carbohydrate metabolism in cirrhotic rat liver. *Exp Toxicol Pathol* 2003;54:270–8.
- Kudryavtseva MV, Emelyanov AV, Sakuta GA, Bezborodkina NN, Kudryavtsev BN. Glycogen-forming function of hepatocytes in the rat regenerating cirrhotic liver after a partial-hepatectomy. *Tissue Cell* 1998;30:1–7.
- Kuwajima M, Golden MS, Katz J, Unger RH, Foster DW, McGarry JD. Active hepatic glycogen synthesis from gluconeogenic precursors despite high tissue levels of fructose 2,6-bisphosphate. *J Biol Chem* 1986;261:2632–7.
- Luck H. Catalase. In: Bergmeyer HU, editor. *Methods of enzymatic analysis*. London: Academic Press; 1971. p. 885–93.
- Magnusson I, Rothman DL, Jucker B, Cline GW, Shulman RG, Shulman GI. Liver glycogen turnover in fed and fasted humans. *Am J Physiol* 1994;266:E796–803.
- Merli M, Eriksson LS, Hagenfeldt L, Wahren J. Splanchnic and leg exchange of free fatty acids in patients with liver cirrhosis. *J Hepatol* 1986;3:348–55.
- Minassian C, Montano S, Mithieux G. Regulatory role of glucose-6-phosphatase in the repletion of the liver glycogen during refeeding in fasted rats. *Biochim Biophys Acta* 1999;1452:172–8.
- Niewoehner CB, Gilboe DP, Nuttall GA, Nuttall FG. Metabolic effects of oral fructose in the liver of fasted rats. *Am J Physiol* 1984a;247:E505–12.
- Niewoehner CB, Gilboe DP, Nuttall FQ. Metabolic effects of oral glucose in the liver of fasted rats. *Am J Physiol* 1984b;246:E89–94.
- Niewoehner CB, Nuttall FQ. Relationship of hepatic glucose uptake to intra-hepatic glucose concentration in fasted rats after glucose load. *Diabetes* 1988;37:1559–66.
- Nishikimi M, Appaji N, Yagi K. The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. *Biochem Biophys Res Commun* 1972;46:849–54.
- Ohgi N, Hirayama C. Vitamin B6 status in cirrhotic patients in relation to apoenzyme of serum alanine aminotransferase. *Clin Biochem* 1988;21:367–70.
- Ohyashiki T, Kamata K, Takeuchi M, Matsui K. Contribution of peroxidation products to oxidative inactivation of rat liver microsomal glucose-6-phosphatase. *J Biochem* 1995;118:508–14.
- Pearce E. *Histochemistry. Theoretical and applied*. Moscow: Foreign Literature Press; 1962.
- Petersen KF, Krssak M, Navarro V, Chandramouli V, Hundal R, Schumann WC, et al. Contributions of net hepatic glycogenolysis and gluconeogenesis to glucose production in cirrhosis. *Am J Physiol* 1999;276:E529–35.
- Planaguma A, Claria J, Miquel R, Lopez-Parra M, Titos E, Masferrer JL, et al. The selective cyclooxygenase-2 inhibitor SC-236 reduces liver fibrosis by mechanisms involving non-parenchymal cell apoptosis and PPARgamma activation. *FASEB J* 2005;19:1120–2.
- Reichen J, Arts F, Schafroth U, Zimmermann A, Heltner Th B, Zysset T. Aminopyrine N-demethylation by rats with liver cirrhosis. *Gastroenterology* 1987;93:719–26.
- Riggio O, Merli M, Leonetti F, Giovannetti P, Foncicello M, Folino S, et al. Impaired nonoxidative glucose metabolism in patients with liver cirrhosis: effects of two insulin doses. *Metabolism* 1997;46:840–3.
- Roach PJ. Glycogen and its metabolism. *Curr Mol Med* 2002;2:101–20.
- Romanova IA, Stalnaya ID. Determination of lipid hydroperoxide with ammonium thiocyanate. In: Orechovich VN, editor. *Modern methods in biochemistry*. Moscow: Medicine; 1977. p. 64–6.
- Rosenfeld EA, Popova IA. *Inborn errors of glycogen metabolism*. Moscow: Meditsina; 1989.
- Schneiter P, Gillet M, Chioloro R, Jequier E, Tappy L. Hepatic nonoxidative disposal of an oral glucose meal in patients with liver cirrhosis. *Metabolism* 1999;48:1260–6.
- Shimamura J. Hepatic key carbohydrate metabolizing enzymes in benign liver disease and hepatocellular carcinoma. *J Med Assoc Okayama* 1987;99:261–72.
- Uchigama M, Michera M. Determination of malonaldehyde precursor in tissues by thiobarbituric acid test. *Anal Biochem* 1978;86:271–8.
- Vardanis A. Particulate glycogen of mammalian liver: specificity in binding phosphorylase and glycogen synthase. *Biochem Cell Biol* 1992;70:523–7.
- Vilkova VA. *Methods of biochemical research*. Leningrad: LGU Press; 1982.
- Weibel ER, Staubli W, Gnagi HR. Correlated morphometric and biochemical studies on the cell. I. Morphometric model, stereologic methods and normal morphometric data for rat liver. *J Cell Biol* 1969;42:68–91.
- Zhao G, Wakabayashi R, Shimoda S, Fukunaga Y, Kumagai M, Tanaka M, et al. Impaired activities of cyclic adenosine monophosphate-responsive element binding protein, protein kinase A and calcium-independent phospholipase A2 are involved in deteriorated regeneration of cirrhotic liver after partial hepatectomy in rats. *Hepatol Res* 2011;41:1110–9.
- Zhan Y-T, Weng J, Li L, Qing Xu Q, Song X, et al. Protective effect of probucol on liver injury induced by carbon tetrachloride in rats. *Hepatol Int* 2011;5:899–905.