

Effects of the 2-ethylthiobenzimidazole hydrobromide (bemithyl) on carbohydrate metabolism in cirrhotic rat liver

MARGARITA V. KUDRYAVTSEVA, NATALIA N. BEZBORODKINA, SERGEY V. OKOVITY, and BORIS N. KUDRYAVTSEY

With 3 figures and 3 tables

Received: April 24, 2002; Revised: November 25, 2002; Accepted: January 28, 2003

Address for correspondence: Dr. MARGARITA V. KUDRYAVTSEVA, Laboratory of Cellular Pathology, Institute of Cytology, Russian Academy of Sciences, Tichoretsky Ave 4, 194064 St. Petersburg, Russia; Tel.: 007 812 2473796, Fax: 007 812 2470341, e-mail: cellpath@mail.cytspb.rssi.ru

Key words: Glycogen; cirrhosis; 2-ethylthiobenzimidazole hydrobromide (bemithyl); glucose-6-phosphatase; glycogen synthase; glycogen phosphorylase.

Summary

The effect of the actoprotector bemithyl (2-ethylthiobenzimidazole hydrobromide) on the content of glycogen and activities of glycogen synthase, glycogen phosphorylase, and glucose-6-phosphatase was studied in the cirrhotic rat liver. The content of glycogen and its fraction was determined by a cytofluorimetric method (KUDRYAVTSEVA et al. 1974). It has been shown that in cirrhosis the content of total glycogen in hepatocytes increases about 3 times and the content of its stable fraction increases 7.5 times. The activity of glucose-6-phosphatase fell to a level as low as 25% of normal. Activities of glycogen synthase and glycogen phosphorylase in the cirrhotic liver did not differ from normal. In the cirrhotic liver, bemithyl produced a decrease of the total glycogen content which was associated with a decrease of the glycogen synthase activity and an increase of the glucose-6-phosphatase and glycogen phosphorylase activities. Thus, the results of our studies indicate a favorable effect of bemithyl on the cirrhotic liver.

Introduction

Chronic hepatitis and cirrhosis of various causes are among diseases that are the most dangerous for human health. Chronic effects of viruses or toxic substances produce a wide spectrum of liver damage. Treatment of these diseases is very difficult due both to the multiple effects of the damaging factors and to the necessity for this organ to perform its numerous functions continuously and effectively.

Various natural or synthetic preparations have been actively used to treat chronic liver lesions. The main criteria of effectiveness of pharmacological preparations used to treat chronic hepatitis and cirrhosis are completeness and rate of recovery of hepatic dysfunction. It has to be admitted, however, that the effectiveness of the available pharmacological preparations (e.g. essential phospholipids and preparations of the animal or plant origin) have turned out to be not as high as was hoped (POROLLO 1980). Therefore, the search for new preparations and study of their mechanisms of action is particularly important.

Recently, to restore physiological function and to treat various structural abnormalities, derivatives of benzimidazole have been used increasingly (SMIRNOV 1994). The benzimidazole derivatives, actoprotectors bemithyl (2-ethylthiobenzimidazole hydrobromide) and ethomazol (5-ethoxy-2-ethylthiobenzimidazole hydrochloride), have been shown to accelerate reparative liver regeneration, to improve function of diseased liver, and to produce immunomodulating and antioxidant effects. It is believed that the mechanism of action of actoprotectors involves activation of the RNA and protein synthesis and that this activation is the most pronounced in organs with a continuous turnover of short-lived proteins (liver, kidney, intestine). Among the liver proteins synthesized intensively under the effect of actoprotectors are enzymes responsible for gluconeogenesis which are of particular importance for maintaining the organs vitality (LOBZIN and SMIRNOV 1993; SMIRNOV 1994).

One of the tissue-specific liver functions essential for the whole organism is glucostatic function. The functional role of the liver is known to depend directly on the state of its glucostatic function. Any lesion of the liver initially involves this function and directly affects glycogen content in hepatocytes.

The goal of this work was to study the effects of 2-ethylthiobenzimidazole hydrobromide on carbohydrate metabolism in the cirrhotic liver.

Materials and methods

The study was carried out in one-year-old male white rats weighting 200–250 g (obtained from the animal nursery "Rappolovo") that were fed once a day at approximately 10 o'clock in the morning. One group of the rats was subjected to chronic exposure to carbon tetrachloride (CCl₄). The rats were intoxicated over 6 months in a special closed chamber of 40 liters volume. They were exposed three times weekly for 15 min to a CCl₄ concentration of 0.05 g/l. As a result, typical liver cirrhosis developed in rats of this group in 6 months. In the second group, the rats were injected with bemithyl (12 mg/kg body weight) 3 times a week for the 1st, 2nd, 4th, and 5th month after the beginning of the CCl₄ poisoning. The rats of the third group received no treatment and served as control. Each group was composed of 5 rats.

To rule out an acute CCl₄ effect, specimens were collected 7 days after the last CCl₄ exposure session. Rats of the control and experimental groups were last fed in the morning, of the day before specimen collection. Liver tissue that was obtained after decapitation of the animals about 10–11 o'clock in the morning was used to perform histological, morphometrical, biochemical, and quantitative cytochemical studies. For histological studies, liver pieces were fixed in 10% neutral formalin, embedded to paraffin, and 5–7- μ m thick sections were cut on a microtome. The sections were stained with hematoxylin-eosin or VAN GIESON (PEARSE 1962). The relative volume of the connective tissue, parenchyma, and other structures in the normal and pathologically altered liver without treatment and after treatment with bemithyl was determined by the method of WEIBEL (1979), using an eye-piece grid, objective 25 \times 0.50, and eye-piece 8 \times . The stained sections were photographed using an Axiophot microscope (Opton, Germany) with objective 10 \times 0.4 and photoocular 1.2 \times .

The content of glycogen and total protein in hepatocytes was determined in smears of isolated cells obtained after the liver perfusion with a 1:1 (v/v) of 0.067 M phosphate buffer and 5% sucrose. Isolated hepatocytes on glass slides were fixed with methanol. Glycogen fractions in hepatocytes were detected using a fluorescent PAS reaction with a reagent of the Schiff type, auramine OO-SO₂ (KUDRYAVTSEVA et al. 1974). Oxidation of cell glycogen was performed with an 0.8% solution of KIO₄ in 0.3% water solution of HNO₃ at pH 2.0 and 20 °C for 90 min. After rinsing in tap and distilled waters, the preparations were placed into a SO₂-saturated (0.2 ml thionyl chloride) 4.5 g/l auramine solution for 40 min (for determination of the labile glycogen fraction) or for 90 min (for total glycogen); the stable glycogen fraction was calculated as difference between labile and total glycogen fractions. The glycogen content in hepato-

cytes was determined using a LUMAM IUV-3 cytofluorimeter (LOMO, St. Petersburg, Russia) The wavelengths 404 and 436 nm, which excite fluorescence of auramine OO (Reanal, Hungary), were isolated from the emission spectrum of a DRSh-250-2 hydrogen lamp with filter FS-1 (8 mm) and SZS-24 (4 mm). Optic glasses ZhZS-19 (1 mm) and ZhS-18 (1 mm) were used as obturating filters to isolate the luminescence light. Photography of isolated hepatocytes stained with auramine-SO₂ was performed using a LUMAM-I-1 luminescence microscope (LOMO, St. Petersburg, Russia) with objectives 90 \times and 20 \times .

The total protein was revealed by staining preparations with Naphthol Yellow S (Sigma) (GAUB et al. 1981). The total protein content in hepatocytes was measured in a Morphoquant scanning absorptional cytophotometer (Carl Zeiss, Jena, Germany), using the wavelength 475 nm, objective 25 \times 0.50, scanning step 0.8 and aperture diameter 0.8 μ m.

The activity of glucose-6-phosphatase in liver homogenates was determined by the method of BERTELOOT et al. (1991); activities of glycogen synthase and glycogen phosphorylase were determined by the methods of VARDANIS (1992). The total protein content was measured by the method of BRADFORD (1976).

Using automatic biochemical analyzers SMA-12/16 (Technicon Instruments Co., USA) and Abbot-Spectrum (Abbot Laboratories, USA), activities of alanine aminotransferase (AlAT) (HENRY et al., 1960) and aspartate aminotransferase (AsAT) (HENRY et al. 1960) and contents of total bilirubin (TB) (GAMBINO and FREDA 1966; JENDRASSIK and GROF 1938), urea nitrogen (UN) (MARSH et al. 1965), creatinine (Cr) (GREENWALD 1928; MARTINES and DOOLAN 1960), and glucose (BROWN 1967) were determined in the blood serum. To evaluate the degree of recovery of hepatic function, a standard duration of hexenal sleep test was performed. This reflects the state of the hepatic microsomal enzyme systems used for metabolizing xenobiotics. Hexenal was injected intraperitoneally at a dose of 80 mg/kg (VERLY 1976).

Statistical treatment of results was performed using standard packets of the PC IBM programs Statgraphics 5.0 and SigmaPlot for Windows 1.0.

Results and discussion

Histological examination of the liver of rats intoxicated for 6 months with CCl₄ revealed development of a typical liver cirrhosis characterized by a marked proliferation of connective tissue, appearance of foci of lymphocytic infiltrates, and disturbances of the lobular structure of the organ (fig. 1a, b). Morphometrical analysis of the normal and cirrhotic liver revealed that in cirrhosis the proportion of parenchyma decreased while that of the connective tissue increased 4 times as compared with normal ($p < 0.001$) (fig. 3). A similar change in the connective tissue and parenchyma has also been observed by other authors (MALLETT et al. 1981; ROJKIND et al. 1983; REICHEN et al. 1987; JAMES et al. 1986).

Injections of bemithyl to the rats chronically intoxicated with CCl₄ led to some decrease of the proportion of connective tissue in the liver. Nevertheless, its relative

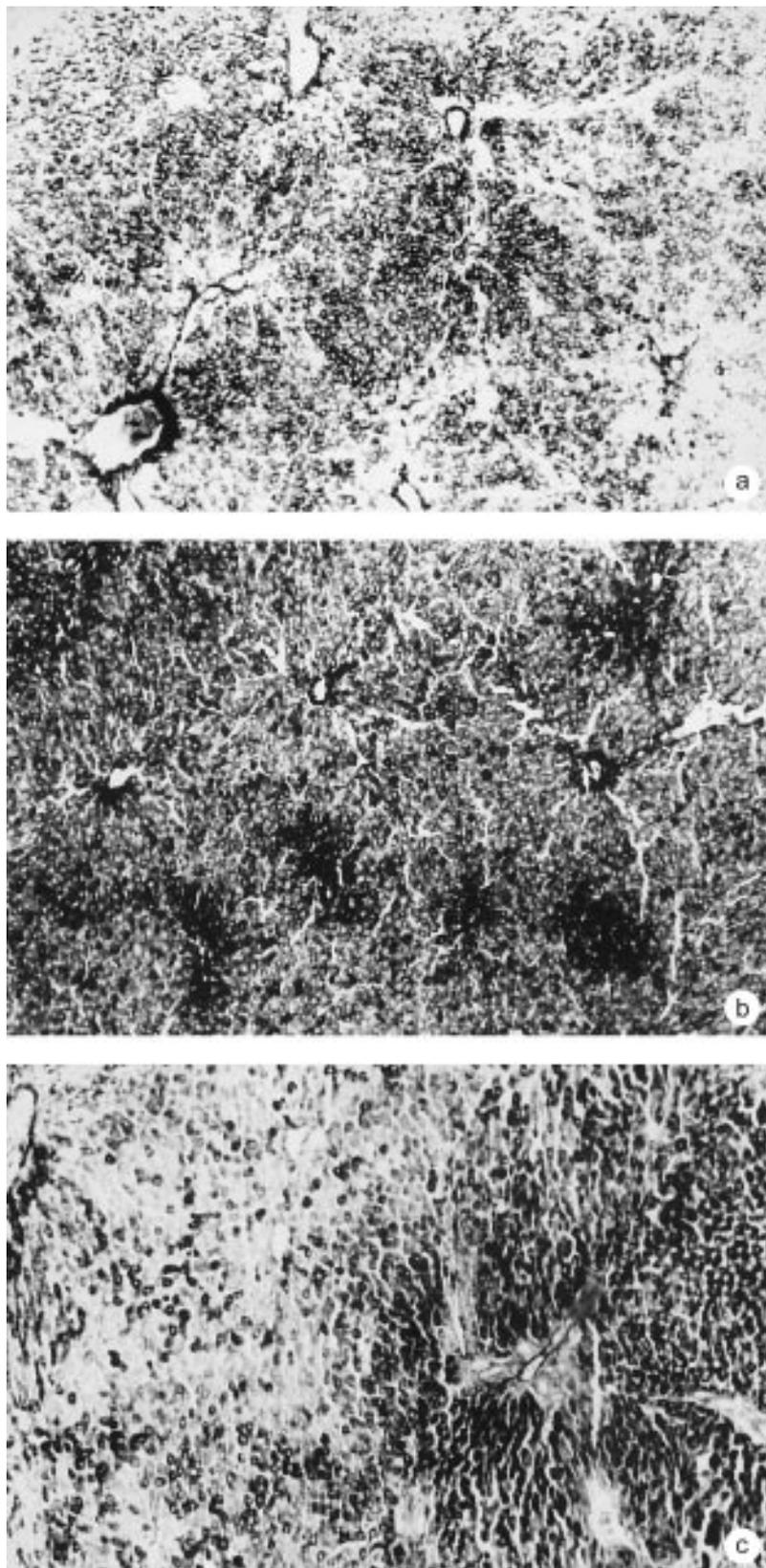


Fig. 1. Sections of the rat liver.
a: normal; **b:** with experimental liver cirrhosis; **c:** with experimental liver cirrhosis after injection of bemithyl. Staining by PAS reaction. Objective 10 \times .

Table 1. Some biochemical parameters of the blood serum in the control and experimental groups of rats.

Group of rats, n = 5	Activity of alanine aminotransferase, nmol/s/l	Activity of aspartate aminotransferase, nmol/s/l	Concentration of total bilirubin, μ M	Concentration of creatinine, μ M	Concentration of urea nitrogen, μ M	Concentration of glucose, μ M
Control (6 months without intoxication)	143.2 \pm 5.2	160.8 \pm 8.1	0.34 \pm 0.04	1.06 \pm 0.10	21.0 \pm 1.3	99.8 \pm 3.4
Liver cirrhosis (6 month of CCl ₄ intoxication)	278.7 \pm 9.1*	228.0 \pm 14.6**	0.58 \pm 0.04**	1.00 \pm 0.04	16.3 \pm 1.2**	98.5 \pm 1.8
Liver cirrhosis + bemithyl (6 month of CCl ₄ intoxication with bemithyl injections)	236.2 \pm 8.6*	186.2 \pm 9.1***	0.44 \pm 0.06***	1.62 \pm 0.40***	26.2 \pm 1.7***	68.8 \pm 3.3*

Mean values were significantly different from those in the control group. *P < 0.001, **P < 0.01, ***P < 0.05.

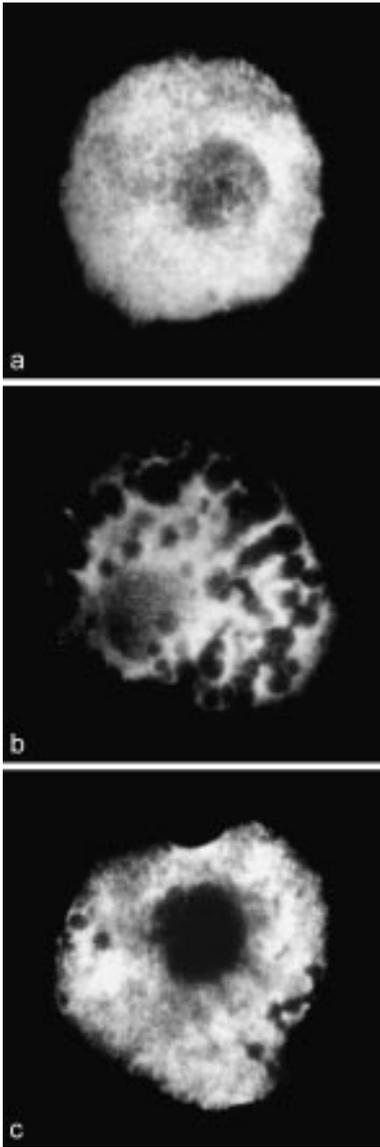


Fig. 2. Hepatocytes of rats. **a:** normal; **b:** with experimental liver cirrhosis; **c:** with experimental liver cirrhosis after injection of bemithyl. Staining by the fluorescent PAS reaction. Objective 90 \times , eye-piece 3 \times .

amount exceeded normal by about twofold ($p < 0.001$) (fig. 3). In addition, the liver structure in the rats treated with bemithyl was characterized by the disappearance of lymphocytic infiltrates and a decrease of the number of Kupffer cells, thus indicating a tendency for recovery of the liver normal structure. However, complete restoration of the normal organ structure was not achieved (fig. 1c).

After performance of the fluorescent PAS reaction, hepatocytes in normal liver were characterized by a rather fine structure and clear contours of the nucleus and cytoplasm (fig. 2a). In the cirrhotic liver, a polymorphism of the cells was observed both for their size and shape and for their structure: there were many vacuolized hepatocytes with blurred contours of the nucleus and cytoplasm (fig. 2b). The structure of hepatocytes of the rats injected with bemithyl was close to normal (fig. 2c).

As seen from table 1, liver cirrhosis in rats was accompanied by an elevation of the ALAT and AsAT activities and of the total bilirubin concentration in the blood. Duration of hexenal sleep in cirrhosis increased about 5 times as compared with normal ($p < 0.001$). This indicates a decrease of activity of the microsomal xenobiotic metabolizing enzyme systems, including hexenal. All these data lead to the conclusion that cirrhosis produces not only damage and death of hepatocytes, but also disturbance of various liver functions.

One of the major functions of liver is its maintenance of a constant blood glucose level. D-glucose represents a transport form of carbohydrates and is the only source of energy used in several tissues. Although some authors have revealed a deviation of the blood glucose concentration from normal in cirrhosis (KRAHENBUHL et al. 1991; GIARDINA et al. 1994), most of the available data indicate that the fasting glucose concentration in the blood serum of patients or rats with liver cirrhosis does not differ from normal (LEATHERDALE et al. 1980; RIGGIO et al. 1982; OWEN et al. 1981; MARCHESINI et al. 1981; CAVALLO-PERIN et al. 1985; PINIEWSKA et al. 1986; JOHANSSON et al. 1994; PETERSEN et al. 1999). The blood sugar level after overnight fasting in cirrhotic patients is believed to decrease only in severe hepatic failure (KRUSZYNSKA and MCINTYRE 1991). This is confirmed by our data that indicate that glucose levels in cirrhosis

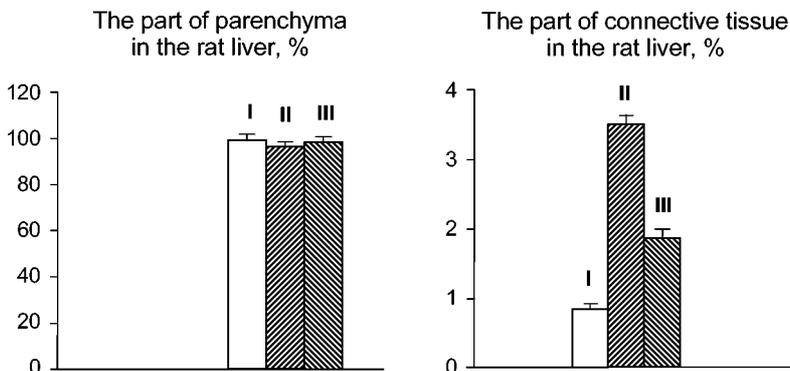


Fig. 3. Values for parenchyma and connective tissue of normal rat liver, in rats with experimental liver cirrhosis and in rats with experimental liver cirrhosis after injection of bemithyl.

I: control, II: liver cirrhosis (6 months of CCl_4 intoxication), III: liver cirrhosis + bemithyl (6 months of CCl_4 intoxication with injections of bemithyl).

do not differ from normal (table 1). At the same time, the data presented in table 1 indicate that bemithyl decreases blood glucose by approximately 30%. A decrease of blood glucose concentration by bemithyl in conjunction with liver regenerating after a partial hepatectomy was earlier reported by GAIVORONSKAYA (2000).

Blood glucose concentration is known to depend not only on the ability of the liver to absorb or produce glucose, but also on the capability of peripheral tissues for its utilization. Among these tissues, a major role in the oxidative and non-oxidative utilization of glucose is played by skeletal muscles which account for 40–45% of the weight of all visceral organs (MUNRO 1969). In liver cirrhosis, the utilization of glucose by skeletal muscles has been shown to decrease markedly (KRUSZYNSKA and MCINTYRE 1991; GIARDINA et al. 1994; JOHANSSON et al. 1994; RIGGIO et al. 1997). An elevation of creatinine and urea nitrogen concentrations in blood (table 1) after injections of bemithyl to rats with liver cirrhosis indicates an increase of the level of protein metabolism in skeletal muscles. The intensification of protein metabolism, which is characteristic of actoprotector effect (SMIRNOV 1993), seems to lead to an increase of glucose utilization in the muscle tissue and, as a result, to a decrease of its blood concentration.

The liver performs its glucostatic function owing to its ability to synthesize or degrade glycogen according to the needs of the organism, as well as via gluconeogenesis. Therefore, evaluation of this liver function can be based on determinations of: 1) the activity of the key enzyme of gluconeogenesis, glucose-6-phosphatase; 2) on the glycogen level in hepatocytes; and 3) on activities of glycogen synthase and glycogen phosphorylase.

Results of the determination of the content of total glycogen and its fractions in the normal and cirrhotic liver are presented in table 2.

In liver cirrhosis, the total glycogen content rises 2–3 times. The labile glycogen fraction is increased twofold. The stable glycogen fraction that normally accounts for about 15–17% of the total glycogen content rises 7.5 times in liver cirrhosis.

All other things being equal, the cell glycogen content is determined by cell size (NEWSHOLME and START 1977).

It has been shown that in cirrhosis the hepatocyte size increases due to a rise of cell ploidy and hypertrophy of cytoplasm (KUDRYAVTSEVA 1991; SAKUTA and KUDRYAVTSEV 1996). Therefore, in the present work the glycogen content was determined not only per cell, but also per protein content in cells. As seen from table 2, in cirrhosis the glycogen content per hepatocyte increased to approximately 3.2 times normal. When taking into account the cell sizes, however, this increase was less pronounced at 2.9 times ($p < 0.001$). The data obtained showed that in cirrhosis not only the content of glycogen changes in cells, but also its structure. In this case the ratio of the labile to stable glycogen fractions changes (table 2).

The data presented in table 2 confirm results of our earlier works on the cirrhotic human and rat liver, which also revealed a rise of glycogen levels (KUDRYAVTSEVA 1987; KUDRYAVTSEVA et al. 1994, 1996, 1998, 2000). However, some authors report a decrease of glycogen levels in the cirrhotic liver (OWEN et al. 1981; KRAHENBUHL et al. 1991, 1996; PETERSEN et al. 1999). The reasons for the discrepancy between our results and the data obtained by these authors are not yet known. It cannot be ruled out that the differences are due to different methodical approaches used for determinations of the liver glycogen levels. In our study, the glycogen content was measured by a quantitative cytochemical method directly in the hepatocytes. This approach is not confounded by heterogeneity of the studied tissue. In the works by others referred to above, the liver glycogen concentration was determined by biochemical methods. When using these, the final result might be affected by heterogeneity of the liver cellular composition that is significantly changed in cirrhosis.

As seen from table 2, the glycogen content in hepatocytes of rats of the experimental group administered with bemithyl exceeded the normal level by as little as 22% ($p < 0.001$). It should be noted that the glycogen fraction ratio in the liver of rats of this group was closer to normal than that of the rats not injected with bemithyl. The decrease of glycogen levels and of the stable glycogen fraction in the liver of the rats treated with bemithyl definitely indicates a favorable effect of this drug on the pathologically altered organ.

Table 2. Content of total glycogen, its labile fraction and its stable fraction in the livers of normal rats and those with liver cirrhosis either untreated or injected with bemithyl.

Group of rats	Total glycogen	Labile fraction of glycogen	Stable fraction of glycogen
Control (6 months without intoxication)	2.39 ± 0.06	1.93 ± 0.05	0.38 ± 0.03
Liver cirrhosis (6 month of CCl ₄ intoxication)	7.73 ± 0.07***	4.53 ± 0.07***	3.05 ± 0.02***
Liver cirrhosis + bemithyl (6 month of CCl ₄ intoxication with bemithyl injections)	2.94 ± 0.05***	1.99 ± 0.06	0.62 ± 0.02***

Mean values were significantly different from those in the control group: *** $P < 0.001$.

The glycogen level in hepatocytes is determined by the ratio of its synthesis and degradation. Any change of the rates of these processes leads either to an increase or to a decrease of the cell glycogen.

It has been shown that the capability of the human and animal cirrhotic liver for postprandial glycogen accumulation is markedly reduced (KRAHENBUHL et al. 1991; GIARDINA et al. 1994; PETERSEN et al. 1999). However, the data about glycogen degradation in the cirrhotic liver are not unanimous. On one hand, a faster glycogen degradation was reported in cirrhosis (KRAHENBUHL et al. 1991). But on the other hand, the glycogenolysis rate in liver of patients with cirrhosis was revealed to be lower by 66% than in normal liver (PETERSEN et al. 1999).

The data about activities of key enzymes of glycogenesis and glycogenolysis, glycogen synthase and glycogen phosphorylase, also are scarce and controversial. Using the model of thioacetamide liver cirrhosis, GIARDINA et al. (1994) have shown a decrease of glycogen synthase activity in the rat cirrhotic liver. The authors believe that this decrease might be the cause of the reduced rate of glycogen accumulation in the cirrhotic liver when the fasting animals started getting food. A significant fall of the total and active forms of glycogen synthase, which correlated with a decrease of the number of hepatocytes, was observed in rats with biliary liver cirrhosis produced by ligation of the bile duct (KRAHENBUHL et al. 1996). On the other hand, the same authors found that the total glycogen synthase level rise in the CCl₄-model of cirrhosis in the rat, while the active form of the enzyme remained unchanged as compared with intact liver (KRAHENBUHL et al. 1991).

The results of our determinations of glycogen synthase activity in normal and cirrhotic rat liver (table 3) indicate that cirrhosis does not affect this enzyme activity. Earlier, we also had revealed that the glycogen synthase activity in the cirrhotic rat liver does not differ from normal (KUDRYAVTSEVA et al. 1994, 1996, 1998). Meanwhile, multiple bemithyl injections to rats intoxicated chronically with CCl₄ led to a decrease by 30% of the glycogen synthase activity in the cirrhotic rat liver as compared with normal ($p < 0.05$) (table 3).

The data about glycogen phosphorylase in the cirrhotically altered liver are similarly scarce and uncertain. KRAHENBUHL et al. (1991, 1996) have found no differences in the total and active forms of glycogen phosphorylase in the rat liver with CCl₄ cirrhosis as compared with control values. However, in the case of biliary liver cirrhosis the same authors have revealed a decrease of both total and active glycogen phosphorylase. Also, a 10% decrease of glycogen phosphorylase was observed in liver of 3 (out of 35) children with chronic liver diseases (PIENIAREK et al. 1985).

Results of determinations of glycogen phosphorylase activity in the liver of our control and experimental rat groups (table 3) indicate that the total enzyme activity in the cirrhotic liver decreased by 38% compared with normal liver ($p < 0.001$). Unlike the total enzyme, activity of glycogen phosphorylase *a* in the cirrhotic liver had no statistically significant difference from normal ($p > 0.05$). Injections of bemithyl to rats submitted to chronic intoxication with CCl₄ resulted in a rise both of the total and active glycogen phosphorylase forms as compared with normal, 1.6 and 1.9 times, respectively (table 3). This might possibly be due to an increase of protein synthesis in the liver.

Although some authors believe that the rate of gluconeogenesis in cirrhosis does not change, or even falls, (STEINER et al. 1962; PINIEWSKA et al., 1986), most available data indicate that gluconeogenesis increases in cirrhotic liver (NEWSHOLME 1976; OWEN et al., 1981, 1983; KRUSZYNSKA and MCINTYRE 1991; KRAHENBUHL and REICHEN 1993; PETERSEN et al. 1999). An enhancement of gluconeogenesis by the cirrhotic liver leads to a change in contribution of glycogenolysis and gluconeogenesis to production of glucose. In normal liver after an overnight fasting, 70–80% of glucose are formed via glycogenolysis and the rest is via gluconeogenesis (WAHREN et al. 1972; KRUSZYNSKA and MCINTYRE 1991). In cirrhosis, however, the contribution of gluconeogenesis to glucose production rises to 67–87% (OWEN et al. 1981; PETERSEN et al. 1999).

In livers of patients with cirrhosis a reduced store of glycogen is considered among the main reasons for an

Table 3. Activities of glucose-6-phosphatase (G6Pase), glycogen phosphorylase (GP) and glycogen synthase (GS) (nmol/min per mg protein) in the livers of rats of different groups.

Group of rats	G6Pase	GP, <i>a</i> form	GP, total activity	GS
Control (6 months without intoxication)	0.77 ± 0.03	1.35 ± 0.05	2.43 ± 0.03	0.0053 ± 0.0004
Liver cirrhosis (6 month of CCl ₄ intoxication)	0.20 ± 0.08***	1.27 ± 0.08	1.51 ± 0.03***	0.0057 ± 0.0005
Liver cirrhosis + bemithyl (6 month of CCl ₄ intoxication with bemithyl injections)	0.59 ± 0.05*	2.52 ± 0.07***	3.79 ± 0.07***	0.0037 ± 0.0006

Mean values were significantly different from those in the control group: * $P < 0.001$, ** $P < 0.01$, *** $P < 0.05$.

intensified shift of cirrhotic liver to metabolism characteristic of a long-term fasting (OWEN et al. 1981, 1983; KRAHENBUHL et al. 1991; KRAHENBUHL and REICHEN 1993). Our results do not confirm this conclusion. We have established (table 2) that the glycogen content in cirrhotic liver cells not only does not decrease, but, on the contrary, rises significantly. Also, it has been shown that activities of the key enzymes of glycogen metabolism, glycogen synthase and glycogen phosphorylase, essentially do not change in cirrhosis (table 3). This indicates no or only slight changes of glycogen metabolism in the cirrhotic liver. Similar data were obtained in our earlier studies (KUDRYAVTSEVA 1987; KUDRYAVTSEVA et al. 1989, 1994, 1996, 1998, 2000).

It was shown earlier that hereditary glycogenosis, type I (von GIERKE's disease), which is due to the lack of glucose-6-phosphatase activity, is usually accompanied by an accumulation of glycogen in human liver and other organs (ROZENFELD and POPOVA 1989). Glucose-6-phosphatase catalyzes detachment of the phosphate group from glucose-6-phosphate formed at glycogenolysis or gluconeogenesis. As a result, free glucose is released to circulation (ARION et al. 1983; WADDEL and BURCHELL 1991). In spite of rather scarce data about activity of glucose-6-phosphatase in cirrhotic liver, a decrease of this enzyme activity in cirrhosis has been shown convincingly (OWEN et al. 1981; PIENIAREK et al. 1985; SOTANIEMI et al. 1985; SHIMAMURA 1987; TAKETA et al. 1988; KRUSZYNSKA and MCINTYRE 1991).

As seen from table 3, the results of our work also indicate a significant decrease of glucose-6-phosphatase activity in the cirrhotic rat liver. The enzyme activity in this case amounts to as low as 25% of that in the control rat liver. This result confirms data of our earlier studies that showed the glucose-6-phosphatase activity in the cirrhotic human and rat liver fell by several fold (KUDRYAVTSEVA et al. 1992, 1994, 1996, 1998). Administration of bemithyl to rats submitted to intoxication with CCl₄ led to an increase of this enzyme activity, although it did not reach the normal level (table 3).

The causes of such a dramatic fall of the glucose-6-phosphatase activity in the cirrhotic liver are unclear. It cannot be explained by a decrease of the proportion of parenchyma in cirrhosis, as it amounted to about 5% compared to normal. It is believed that one of important causes capable of leading to the decrease of the glucose-6-phosphatase system is a disturbance of the cellular endoplasmic reticulum as a result of a damaging effect of various lipid peroxide products (GHOSHAL and RECKNAGEL 1965; CIGNOLI and CASTRO 1971; CASTRO et al. 1973; BENEDETTI et al. 1977, 1980; KOSTER and SLEE 1980; de GROOT et al. 1985; OHYASHIKI et al. 1995). Therefore, a decrease of the superoxide dismutase and catalase activities ($p < 0.05$) and accordingly an increase of MDA levels ($p < 0.05$) in rats with liver cirrhosis in our experiments can indicate that an enhancement of lipid peroxidation is one of the main mechanisms causing the fall of activity of glucose-6-phosphatase in CCl₄-induced cirrhosis.

In conclusion, the data obtained allow the conclusion that the cirrhotic transformation of liver parenchyma leads to an almost 3-fold accumulation of glycogen in hepatocytes. Such elevation of glycogen concentration in cells does not seem to be due to changes of activities of the key enzymes of glycogen metabolism, since the level of active forms of these enzyme in cirrhosis in fact remains unchanged. The main reason for the rise of glycogen levels in hepatocytes of cirrhotic liver is a pronounced fall in glucose-6-phosphatase activity due to lipid peroxidation in membranes of endoplasmic reticulum. The fall of glycogen levels in hepatocytes after injections of bemithyl to rats subjected to chronic CCl₄ intoxication seems to be due to a complex of causes, such as a decrease of glycogen synthase as well as an increase of activities of glucose-6-phosphatase and glycogen phosphorylase. The results of the present work have also shown that bemithyl promotes recovery of the glycogen forming function of hepatocytes of cirrhotic liver.

Acknowledgment: The work was supported by Russian Fund of Fundamental Investigation (RFFI No. 01-04-49115, RFFI No. 02-04-06714).

References

- ARION WJ, SCHULZ LO, LANGE AJ, et al.: The characteristics of liver glucose-6-phosphatase in the envelope of isolated nuclei and microsomes are identical. *J Biol Chem* 1983; **258**: 12661–12669.
- BENEDETTI A, CASINI AF, FERRALI M, et al.: Studies on the relationship between carbon tetrachloride-induced alterations of liver microsomal lipids and impairment of glucose-6-phosphatase activity. *Exp Mol Pathol* 1977; **27**: 309–323.
- BENEDETTI A, COMPORTI M, ESTERBAUER H: Identification of 4-hydroxynonenal as a cytotoxic product originating from the peroxidation of liver microsomal lipids. *Biochim Biophys Acta* 1980; **620**: 281–296.
- BERTELOOT A, VIDAL H, WERWE G: Rapid kinetics of liver microsomal glucose-6-phosphatase. *J Biol Chem* 1991; **266**: 5497–5504.
- BROWN ME: Ultra-micro sugar determination using 2,9-dimethyl-1,10-phenanthroline hydrochloride (neocuproine). *Diabetes* 1961; **10**: 60–62.
- CASTRO J, CIGNOLI E, DIAZ GOMES M, et al.: Mecanismo de la hepatotoxicidad de tetracoloruro de carbono, diseno de antidotos a nivel molecular. *Rev Asociac Bioquim Argentina* 1973; **38**: 161–206.
- CAVALLO-PERIN P, CASSADER M, BOZZO C: Mechanism of insulin resistance in human liver cirrhosis. *J Clin Invest* 1985; **75**: 1659–1665.
- CIGNOLI E, CASTRO J: Lipid peroxidation, necrosis and the in vivo depression of liver glucose-6-phosphatase by carbon tetrachloride. *Exp Mol Pathol* 1971; **14**: 43–56.
- de GROOT H, NOLL T, TOLL T: Loss of latent activity of liver microsomal membrane enzymes evoked by lipid peroxidation. Studies of nucleoside diphosphatase, glucose-6-phosphatase, and UDP glucuronyltransferase. *Biochim Biophys Acta* 1985; **815**: 91–96.

- GAIVORONSKAYA VV, OKOVITY SV, SHUSTOV EB: Effect of bemithyl, etomerzol, and yakton on processes of liver regeneration after a partial hepatectomy. *Eksper Klin Farmakol* 2000; **63**: 34–36.
- GAMBINO SR, FRED A VJ: The measurement of amniotic fluid bilirubin by the method of Jendrassik and Grof. *Amer J Clin Pathol* 1966; **46**: 198–203.
- GAUB J, FANERHOLDT L, KEIDING S, et al.: Cytophotometry of liver cells from ethanol-fed rat: ethanol causes increased polyploidization and protein accumulation. *Eur J Clin Invest* 1981; **11**: 235–237.
- GHOSHAL A, RECKNAGEL R: On the mechanism of carbon tetrachloride hepatotoxicity: co-occurrence of loss G-6P-ase activity with peroxidation of microsomal lipid. *Life Sci* 1965; **4**: 2195–2209.
- 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**: 900–906.
- GREENWALD I: The chemistry of Jaffe's reaction for creatinine via the isolation of the red compound. *J Biol Chem* 1928; **80**: 103–106.
- HENRY RJ, CHIAMORI N, GOLUB OJ, et al.: Revised spectrophotometric methods for the determination of glutamic-oxalacetic transaminase, glutamic-pyruvic transaminase, and lactic acid dehydrogenase. *Tech Bull Reg Med Tech* 1960; **30**: 149–166.
- JENDRASSIK L, GROF P: Vereinfachte photometrische Methoden Zur Bestimmung des Blutbilirubins *Biochem Z* 1938; **297**: 81–89.
- JOHANSSON U, ERIKSSON LS, GALUSKA D, et al.: Insulin action on glucose transport in isolated skeletal muscle from patients with liver cirrhosis. *Scand J Gastroenterol* 1994; **29**: 71–76.
- KOSTER JF, SLEERG: Lipid peroxidation of rat liver microsomes. *Biochim Biophys Acta* 1980; **620**: 489–499.
- KRAHENBUHL L, TALOS C, REICHEN J, et al.: Progressive decrease in tissue glycogen content in rats with long-term cholestasis. *Hepatology* 1996; **24**: 902–907.
- KRAHENBUHL S, WEBER FL, BRASS EP: Decreased hepatic glycogen content and accelerated response to starvation in rats with carbon tetrachloride-induced cirrhosis. *Hepatology* 1991; **14**: 1189–1195.
- KRAHENBUHL S, REICHEN J: Decreased hepatic glucose production in rats with carbon tetrachloride-induced cirrhosis. *J Hepatol* 1993; **19**: 64–70.
- KRUSZYNSKA Y, MCINTYRE N: Carbohydrate metabolism. In: *Oxford textbook of clinical hepatology*. Oxford: Oxford University Press, 1991; 129–143.
- KUDRYAVTSEV BN: Cellular mechanisms of the normal and regenerative growth of mammalian liver. Doctorate Dissertation. 1991.
- KUDRYAVTSEVA MV: Glycogenesis of liver cells in chronic hepatitis and its diagnostic significance. *Usp Hepatol* 1987; **13**: 201–211.
- KUDRYAVTSEVA MV, BEZBORODKINA NN, KUDRYAVTSEV BN: Glycogen-forming function of hepatocytes in cirrhotically altered rat liver after treatment the chorionic gonadotropin. *Exp Toxic Pathol* 2001; **53**: 57–63.
- KUDRYAVTSEVA MV, BEZBORODKINA NN, KUDRYAVTSEV BN: Restoration of the glycogen-forming function of hepatocytes in cirrhotically altered rat liver after treatment of the chorionic gonadotropin. *Exp Toxic Pathol* 2001; **53**: 57–63.
- KUDRYAVTSEVA MV, EMEL'YANOV AV, SAKUTA GA, et al.: Glycogen-forming function of hepatocytes in the rat regenerating cirrhotic liver after a partial hepatectomy. *Tissue and Cell* 1998; **30**: 261–267.
- KUDRYAVTSEVA MV, EMEL'YANOV AV, SAKUTA GA, et al.: Glycogen-forming function of hepatocytes under conditions of regeneration of rat cirrhotic liver after a partial hepatectomy. *Cytologiya* 1996; **38**: 934–948.
- KUDRYAVTSEVA MV, EMEL'YANOV AV, SAKUTA GA, et al.: Cytofluorimetric study of the content of glycogen and its fractions in hepatocytes of patients with liver cirrhosis of different etiologies. *Tsitologiya* 1992; **34**: 100–107.
- KUDRYAVTSEVA MV, KUDRYAVTSEV BN, ROZANOV Yu M: On two glycogen fractions in rat liver cells (cytofluorimetric study). *Tsitologiya* 1974; **16**: 851–858.
- LEATHERDALE BA, CHASE RA, ROGERS J, et al.: Forearm glucose uptake in cirrhosis and its relationship to glucose tolerance. *Clin Sci* 1980; **59**: 191–198.
- LOBZIN YuV, SMIRNOV AV: Bemithyl in complex therapy and rehabilitation of patients with viral hepatitis A. In: *Physiologically active substances. Interinstitution collection of scientific papers*. 1993; **25**: 23–27.
- MALLET L, POLIOT A, AMAL D, et al.: Degree of hepatic enzyme induction might be related to the amount of fibrosis in rats poisoned by carbon tetrachloride. *Gastroenterol Clin Biol* 1981; **5**: 572–576.
- MARCHESINI G, ZOLI M, ANGLOLINI A, et al.: Muscle protein breakdown in liver cirrhosis and the role of altered carbohydrate metabolism. *Hepatology* 1981; **1**: 294–299.
- MARSH WH, FINGERHUT B, MILLER H: Automated and manual direct methods for the determination of blood urea. *Clin Chem* 1965; **11**: 624–627.
- MARTINES E, DOOLAN PD: Determination of creatinine in small quantities of plasma. Observations on two methods. *Clin Chem* 1960; **6**: 233–242.
- MUNRO HN: Evolution of protein metabolism in mammals. In: *Mammalian protein metabolism*. New York: Academic Press, 1969; **3**: 133–182.
- NEWSHOLME E, START K: Regulation of metabolism. Moscow, "Mir", 1977; 343 pp.
- OHYASHIKI T, KAMATA K, TAKEUCHI M, et al.: Contribution of peroxidation products to oxidative inactivation of rat liver microsomal glucose-6-phosphatase. *J Biochem* 1995; **118**: 508–514.
- OWEN OE, REICHLER FA, MOZZOLI JMA, et al.: Hepatic, gut, and renal substrate flux rates in patients with hepatic cirrhosis. *J Clin Invest* 1981; **68**: 240–252.
- OWEN OE, TRAPP VE, REICHARD GA, et al.: Nature and quantity of fuels consumed in patients with alcoholic cirrhosis. *J Clin Invest* 1983; **72**: 1821–1832.
- PEARSE E: *Histochemistry, Theoretical and Applied*. Moscow, "Inostrannaya Literatura", 1962; 962 pp.
- PETERSEN KF, KRSSAK M, NAVARRO V, et al.: Contributions of net hepatic glycogenolysis and gluconeogenesis to glucose production in cirrhosis. *Am J Physiol* 1999; **276**: E529–535.
- PIENIAREK D, PRONICKA E, CABALSKA B, et al.: Glikogenoza typu VI i VIa. *Pediat Pol* 1985; **60**: 489–496.
- PINIEWSKA DM, MCCULLOCH AJ, BRAMBLE MG, et al.: Glucose turnover in compensated hepatic cirrhosis. *Horm Met Res* 1986; **18**: 834–837.
- POROLLO VI: Pharmacological stimulation of regeneration processes with pyrimidine derivatives and molecular mechanisms of their action. Doctorate Dissertation. 1980.

- REICHEN J, ARTS F, SCHAFROTH U, et al.: Aminopyrine N-Demethylation by rats with liver cirrhosis. *Gastroenterology* 1987; **93**: 719–726.
- RIGGIO O, MERLI M, CANGIANO C, et al.: Glucose intolerance in liver cirrhosis. *Metabolism* 1982; **31**: 627–634.
- ROJKIND M, ROJKIND MH, CORDERO-HERNANDEZ J: In vivo collagen synthesis and deposit in fibrotic and regeneration rat liver. *Collagen Rel Res* 1983; **3**: 335–347.
- ROZENFELD EL, POPOVA IA: Congenital Disturbances of Glycogen Metabolism. Moscow, "Medicine", 1989; 240 pp.
- SAKUTA GA, KUDRYAVTSEV BN: Cellular mechanisms of regeneration of rat cirrotic liver. I. Correlation of processes of cell proliferation, polyploidization, and hypertrophy after cessation of chronic CCl₄ Action. *Tsitologiya* 1996; **38**: 1158–1171.
- SHIMAMURA J: Hepatic key carbohydrate metabolizing enzymes in benign liver disease and hepatocellular carcinoma. *J Med Assoc Okayama* 1987; **99**: 261–272.
- SMIRNOV AV: Bemithyl: mechanism of action and related effects. *Physiologically Active Substances. Interinstitution Collection of Scientific Works. Kiev: Naukova Dumka*; 1993.
- SMIRNOV AV: Peculiarities of actoprotectors as an independent pharmacological class. In: *Antihypoxants and Actoprotectors: Results and perspectives. St. Petersburg, 1994*; **3**: 164.
- SOTANIEMI EA, SAAMI HU, ARRANTO AJ, et al.: Carbohydrate intolerance associated with reduced hepatic glucose phosphorylating and releasing enzyme activities and peripheral insulin resistance in alcoholics with liver cirrhosis. *J Hepatol* 1985; **1**: 277–287.
- STEINER JW, CARRUTHERS JS, KALIFAT SR: Observations on the fine structure of rat liver cells in extrahepatic cholestasis. *Z Zellforsch* 1962; **59**: 564–568.
- TAKETA K, TANAKA A, WATANABE A, et al.: Undifferentiated patterns of key carbohydrate-metabolizing enzymes in injured livers. Acute carbon-tetrachloride intoxication of rat. 1976; **21**: 158–173.
- VARDANIS A: Particulate glycogen of mammalian liver: specificity in binding phosphorylase and glycogen synthase. *Biochem Cell Biol* 1992; **70**: 523–527.
- VERLY WC: *The control of liver growth. New York: Chalmers, 1976*; 112 pp.
- WADDELL ID, BURCHELL A: Transverse topology of glucose-6-phosphatase in rat hepatic endoplasmic reticulum. *Biochem J* 1991; **275**: 133–137.
- WEIBEL ER: *Stereological methods. London: Academic Press, 1979*; 245 pp.