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Glycoprotein Metabolism in Dimethylnitrosamine Induced Hepatic Fibrosis in Rats

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Glycoproteins play a major role in the pathogenesis of hepatic fibrosis by accumulating in the sinusoids and the space of Disse. In order to obtain more information about the altered metabolism of glycoproteins during the development of human hepatic fibrosis, the carbohydrate moieties of the glycoproteins were monitored in experimentally induced hepatic fibrosis. The liver injury was induced by injecting dimethylnitrosamine intraperitoneally in male albino rats. The injections were given on the first 3 consecutive days of each week over a period of 21 days. Glycoprotein moieties such as total hexose, hexosamine, fucose and sialic acid were estimated in liver, serum and urine samples on days 7, 14 and 21 of the experiment. The results indicated a significant decrease in total hexose and an increase in fucose levels in the liver tissue during dimethylnitrosamine administration. While protein bound hexose in the serum showed a significant decrease, sialic acid levels were notably increased. The other glycoprotein moieties both in liver and serum also showed an increase in the later periods of study, especially on day 21. All glycoprotein moieties exhibited a significant increase in the rate of urinary excretion on the 14th and 21st days, indicating an increased rate of metabolic degradation in the acute phase of hepatic fibrosis. The results suggest that glycoproteins undergo changes in both synthesis and the degradation during hepatic fibrosis. The relative alterations in these processes will play a vital role in determining the progression of hepatic fibrosis.

Keywords: Glycoprotein Dimethylnitrosamine Hepatic fibrosis Fucose Sialic acid

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INTRODUCTION

Glycoproteins are present in the extracellular matrix and are believed to contribute to the structure of the matrix (Labat-Robert and Robert, 1988). Fibronectin and laminin are the principal structural glycoproteins present in the liver (Rojkind and Perez-Tamayo, 1983; Plebani and Burlina, 1991). The other major glycoproteins in the liver include vitronectin, entactin and undulin (Tomihira, 1991; Preissner, 1991; Bender *et al.*, 1981; Schwoegler *et al.*, 1994; Casini *et al.*, 1994; Milani *et al.*, 1994). Fibronectin binds strongly and specifically to collagen. The interaction of fibronectin with native collagen is physiologically significant in the maintenance of proper tissue organization and providing overall structural stability of the liver (Kleinman *et al.*, 1981). Laminin is a high molecular weight structural glycoprotein localized in the basement membranes. In the liver, laminin closely follows the distribution of type IV collagen (Hahn *et al.*, 1980; Carlsson *et al.*, 1981). Laminin is absent in the sinusoidal lining. Laminin plays an important role in morphogenesis and in the organization of liver plates during regeneration (Carlsson *et al.*, 1981). Gabrielli and Corrocher (1991) reported that laminin and type IV collagen are putative markers of basement membrane formation and sinusoid capillarization; an important pathological process in hepatic fibrosis.

Vitronectin is an important glycoprotein in cell attachment and is produced by all liver cells (Tomihira, 1991). Kobayashi *et al.* (1994) reported that vitronectin content was

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significantly increased in the liver tissue in chronic liver diseases. It was also suggested that vitronectin could be involved in the modulation of fibrogenesis in acute liver injuries (Inuzuka *et al.*, 1994). Entactin is a sulfated glycoprotein having binding sites for laminin, fibronectin, type IV collagen and cell surfaces. Its highest distribution was reported in fat storing cells both in normal and fibrotic liver (Schwoegler *et al.*, 1994). Entactin plays a major role in sinusoidal basement membrane formation during fibrogenesis. Undulin is a large extracellular matrix glycoprotein and it participates in the reorganization of connective tissue during hepatic fibrosis (Casini *et al.*, 1994). It was reported that undulin deposition and gene expression were enhanced in fibrotic liver (Milani *et al.*, 1994).

The carbohydrate moieties of glycoproteins include neutral sugars, amino sugars and sialic acid. Even though much data is available on biochemical changes and connective tissue metabolism in hepatic fibrosis, very little investigation has been carried out with respect to glycoprotein metabolism. It has been suggested that abnormal connective tissue metabolism plays a vital role in the development of hepatic fibrosis (Rojkind, 1982). Lombart *et al.* (1980) reported that the activity of liver glycosyltransferases and the rate of transfer of N-acetylglucosamine, galactose and sialic acid to sugar depleted glycoproteins were increased during inflammation of liver. The increased glycosylation potential of the liver may play an important role in the secretion of glycoproteins. Increased levels of dolichol phosphokinase activity, dolichol phosphate linked glycosylation of mannose and glucose in the inflamed liver of rats have also been reported (Coolbear and Mookerjea, 1981).

In the present study, an animal model was selected for effective monitoring of glycoprotein metabolism during the progression of hepatic fibrosis. It was shown that dimethylnitrosamine (DMN) induced hepatic fibrosis in rats is a good reproducible model for studying biochemical and pathophysiological changes associated with the development of hepatic fibrosis and alcoholic cirrhosis of human beings (Jenkins *et al.*, 1985; Jezequel *et al.*, 1987; Jezequel *et al.*, 1989). Since the pathogenic process of hepatic fibrosis and cirrhosis leads to extreme necrosis and regeneration of the liver tissue which results in the distortion of the lobular architecture, studies on the alterations in the metabolism of structural macromolecular components such as collagen, glycoprotein and glycosaminoglycan are of considerable relevance to the incidence of hepatic fibrosis and cirrhosis. Hence, in the present investigation we have studied the metabolic alteration of important glycoprotein moieties such as hexose, hexosamine, fucose and sialic acid in the liver and serum during the progression of DMN induced hepatic fibrosis in rats. Quantification of glycoprotein moieties was also carried out in the urine samples in order to obtain a clear picture of the metabolic degradation of glycoproteins during hepatic fibrosis.

MATERIALS AND METHODS

Chemicals

Dimethylnitrosamine, *p*-dimethylaminobenzaldehyde, Dowex-50W, thiobarbituric acid, glucosamine hydrochloride, fucose and N-acetylneuraminic acid were purchased from Sigma Chemical Company, U.S.A. Acetylacetone was procured from Fluka AG, Switzerland and sodium metaperiodate from Budapest, Hungary. Sodium arsenite was obtained from J. T. Baker Chemical Co., Phillipsburg, U.S.A. All other chemicals used were of analytical grade.

Animals

Adult male albino rats of Wistar strain at the age of about 3 months and weighing between 180 and 200 g were used. The animals were bred and maintained in the animal house with commercial rat feed (Hindustan Lever Ltd, Bombay, India) and water *ad libitum*. They were housed in polypropylene cages with a wire mesh top and a hygienic bed of husk.

Induction of hepatic fibrosis

Hepatic fibrosis was induced by i.p. injections of dimethylnitrosamine (DMN) in doses of 1 μl (diluted to 1 : 100 with 0.15 M sterile NaCl)/100 g body weight. The injections were given on the first 3 consecutive days of each week over a period of 21 days. Control animals also received an equal volume of 0.15 M NaCl without DMN. The injections were given without anesthesia. For urine collection, the animals were transferred to metabolic cages 24 hr prior to sacrifice. Urine was collected under a layer of toluene and stored at 4°C until analyzed.

Treated animals were sacrificed on days 7, 14 and 21 from the beginning of exposure. Some of

the control animals were sacrificed at the beginning of the experiment and some together with the treated animals on days 7, 14 and 21, and the pooled value was used as control. The control and the 7th day group comprised 10 rats each, while the 14th and 21st day group consisted 9 and 7 rats, respectively. All the animals were anesthetized with diethyl ether before sacrifice. Blood was collected from a deep cut made on the right jugular vein on the neck with a scalpel blade. It was allowed to clot at 30°C for 1 hr and the serum was separated by centrifugation at 2000 *g* for 10 min and stored at −20°C. The livers were rapidly removed, rinsed in cold saline and stored at −70°C until analyzed.

Assessment of the degree of hepatic fibrosis

The degree of hepatic fibrosis was evaluated histopathologically as well as by quantifying collagen content in the liver. For histopathology, the sections were stained with hematoxylin and eosin and examined under a Nikon Labophot microscope.

The total collagen content in the liver was determined by the estimation of hydroxyproline, a characteristic imino acid in collagen. For the determination of hydroxyproline, all samples were hydrolyzed in 6 N HCl in sealed tubes at 110°C for 16 hr. The hydrolyzed samples were evaporated to dryness in a boiling water bath to remove acid and the residue was redissolved in distilled water and made up to a known volume. It was treated with activated charcoal and filtered through Whatman filter paper. The clear filtrate was used for the determination of hydroxyproline according to the method of Woessner (1961). The total collagen content in the liver was calculated by multiplying the hydroxyproline content by the factor 7.46, as postulated by Neuman and Logan (1950).

Extraction of liver glycoproteins

The livers were rinsed in ice cold 0.15 M NaCl to remove blood-borne contaminants and the water was blotted off. It was chopped after weighing in an electronic balance and each sample was homogenized separately in a Polytron homogenizer (Kinematica AG, Switzerland) using ice cold 0.15 M NaCl solution at 4°C. The final concentration of the homogenate was adjusted to 100 mg tissue/ml and this was used for the estimation of carbohydrate moieties of glycoproteins.

Determination of total hexose and protein-bound hexose

The total hexose present in the liver homogenate and urine samples was determined by the method of Dubois *et al.* (1956) using phenol–sulfuric acid reagent. The protein-bound hexose present in the serum was determined according to the method described by Winzler (1955) after precipitating the serum with 95% ethanol. The precipitate was dissolved in 0.1 N NaOH and allowed to react with freshly prepared orcinol–sulfuric acid reagent. The optical density was measured at 540 nm using a Shimadzu spectrophotometer.

Analysis and estimation of hexosamine in liver homogenate

The hexosamine content in the liver homogenate was determined according to the method described by Boas (1953). Since large amounts of neutral sugars are present in tissue homogenates, hexosamines were separated from neutral sugars by adsorption and elution from ion-exchange resin (Dowex 50X4, 200–400 dry mesh, H^+ form). Prior to the separation of hexosamines from the ion-exchange column, hexosamines were liberated by acid hydrolysis with 4 N HCl for 8 hr in sealed tubes kept in a boiling water bath. This results in the optimal release of hexosamines from several glycoproteins (Spiro, 1962). The acid from the hydrolysate was completely removed in a HaakeBuchler PE6 rotary evaporator (Haake-Buchler Instruments, Inc., Saddle Brook, New Jersey, U.S.A.) at 50°C prior to placing the samples on ion-exchange columns. The ion-exchange resin was washed prior to use in a Buchner funnel with 2 N NaOH, distilled water, 3 N HCl and finally with distilled water, in that order, and transferred into the column. The sample was loaded on the top of the column and washed with triple distilled water. The hexosamines were then eluted from the column with 10 ml of 2 N HCl and the elute was collected and evaporated to dryness at 50°C in a rotary evaporator. The residue was re-dissolved in distilled water and used for hexosamine estimation employing *p*-dimethyl-aminobenzaldehyde reagent.

Estimation of hexosamine in urine and protein-bound hexosamine in serum

Hexosamine present in the urine was determined by the method of Elson and Morgan

(1933) as adopted by Rimington (1940) after acid hydrolysis with 4 N HCl for 8 hr in sealed tubes at 100°C. The protein-bound hexosamine present in the serum was determined by the method of Winzler (1955) after ethanol precipitation as in the case of hexose. The precipitate was hydrolyzed with 4 N HCl in a boiling water bath with glass marble as condenser for 4 hr. The acid present in the hydrolysate was evaporated in a rotary evaporator at 50°C. The residue was dissolved in distilled water and the hexosamine content was determined using freshly prepared *p*-dimethylaminobenzaldehyde reagent. The optical density was measured at 530 nm in a Shimadzu UV-260 spectrophotometer.

Determination of fucose

The fucose present in the liver homogenate, serum and urine samples was simultaneously determined by the method of Dische and Shettles (1948) using cysteine. All the samples were treated with 85% cold sulfuric acid and vortexed well. The tubes were covered with glass marbles and placed in a vigorously boiling water bath for exactly 3 min. The samples were cooled and treated with 0.1 ml of freshly prepared cysteine reagent. The absorbance was determined after 2 hr at 396 and 427 nm in a Shimadzu UV-160 spectrophotometer using multi λ mode. To avoid hexose interference, the content of fucose in a given sample was determined by subtracting the absorbance difference of the sample at 396 and 427 nm without cysteine from the absorbance difference of the same sample at 396 and 427 nm with cysteine.

Determination of sialic acid

The sialic acid (N-acetylneuraminic acid) present in the liver homogenate, serum and urine was determined by the thiobarbituric acid method described by Warren (1959). Since this assay measures only free sialic acids, all samples were hydrolyzed with 0.1 N H_2SO_4 at 80°C for 1 hr. The hydrolyzed samples were mixed with 0.1 ml of periodate solution (0.2 M sodium metaperiodate in 9 M phosphoric acid) and allowed to stand at room temperature for 20 min. It was treated with arsenite solution (10% sodium arsenite in a solution of 0.5 M sodium sulfate in 0.1 N H_2SO_4) and the tubes were shaken until the yellow–brown color of the liberated iodine disappeared. Finally all the samples were mixed with 3 ml of 0.6% thiobarbituric acid and kept in a boiling water bath for 15 min. The optical density was measured using a spectrophotometer at 549 nm after extracting the developed color into 4 ml of redistilled cylcohexanone.

Urine creatinine was estimated by the method of Bonsnes and Taussky (1945).

Statistical analysis

Arithmetic mean and standard error were calculated for the data. The results were statistically evaluated using one-way analysis of variance (ANOVA). The control mean values were compared with the treated mean values on days 7, 14 and 21 of the experiment using the least significant difference method. The value of $P < 0.01$ was considered as statistically significant. The correlation coefficient was calculated from the data to study the linear relationship between liver, serum and urinary levels of various glycoprotein moieties.

RESULTS

The progression of hepatic fibrosis, monitored by histopathological examination of the liver tissue demonstrated severe centrilobular congestion and marked dilatation of central vein and sinusoids on the 7th day. It also showed hemorrhagic necrosis and mild bile duct hyperplasia. On the 14th day, there was massive centrilobular necrosis and intense neutrophilic infiltration. On the 21st day of DMN treatment, the liver showed many changes which are common in alcoholic fibrosis. There was diffuse centrilobular necrosis in all cases. Hydropic and focal fatty changes were seen. Increased fibrosis with bile duct hyperplasia were noticed consistently. Regeneration of hepatocytes was seen in many cases. The hepatocytes showed Mallory's hyaline within cytoplasm. Apoptosis and dysplasia were frequent. There was bridging necrosis and early fibrosis between portal tract and central vein. On the 21st day, liver sections showed well-developed fibrosis around the central vein.

The total collagen content in the liver during the pathogenesis of DMN induced hepatic fibrosis is demonstrated in Fig. 1. It was observed that by the 21st day of DMN treatment, fibrosis was well developed with a 4-fold increase of total collagen content in the liver.

The results obtained with regard to the effect

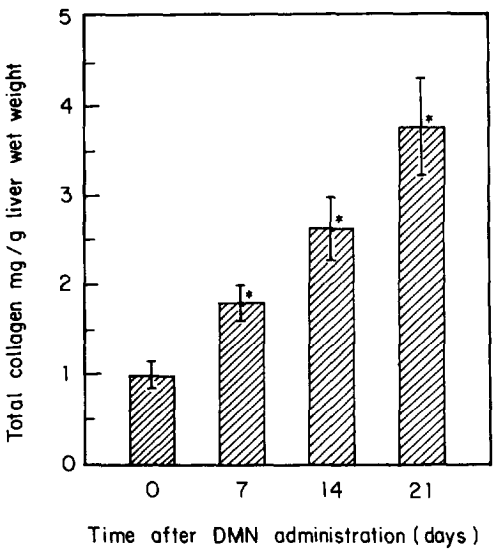

Fig. 1. Total collagen content in the liver during DMN induced hepatic fibrosis in rats (*$P < 0.001$). The values are mean and standard error.

of dimethylnitrosamine on various glycoprotein moieties in rat liver are presented in Table 1. A significant decrease was noticed in total hexose levels in the liver during the course of DMN treatment. In contrast, an increasing trend was observed on all other glycoprotein moieties monitored during hepatic fibrosis. A highly significant increase was noticed on fucose levels from the 7th to the 21st day of DMN treatment. While liver sialic acid increased only on the 21st day of DMN treatment, hexosamine levels showed a significant increase on the 14th and 21st day but not on the 7th day.

The alteration of various glycoprotein moieties in rat serum during the progression of hepatic fibrosis is represented in Table 2. A significant decrease was noticed in the mean serum levels of protein-bound hexose in DMN treated animals. It is interesting to note that the gradual decrease was only up to the 14th day. On the 21st day, the protein bound hexose showed a plateau when compared to the 14th day value. No significant alteration was observed in the mean serum level of protein bound hexosamine during the period of investigation. Regarding serum fucose levels, a significant increase was recorded only on the 21st day. An increase was noticed in serum sialic acid levels from the 7th to the 21st day of DMN treatment.

Table 3 shows the metabolic degradation and urinary excretion of various glycoprotein moieties during the course of DMN induced hepatic fibrosis. All four carbohydrate moieties of glycoproteins, viz. total hexose, hexosamine, fucose and sialic acid, exhibited an increase in the urine levels from the beginning to the 21st day of DMN treatment in systematic fashion. In all four cases, the increase was highly significant on the 14th and 21st days after administration of DMN. But on the 7th day, the difference was not significant for any of the glycoproteins studied. In all cases, the maximum urinary excretion of carbohydrate moieties was recorded on the 21st day. In most cases the

Table 1. Effect of DMN on glycoprotein moieties in rat liver

Glycoprotein moiety	Control ($n = 10$)	Day 7 ($n = 10$)	Day 14 ($n = 9$)	Day 21 ($n = 7$)
Total hexose	2601.10 ± 226.59	1384.42 ± 92.57**	696.78 ± 61.95**	481.77 ± 49.80**
Hexosamine	34.14 ± 1.95	39.29 ± 1.02	41.06 ± 1.30*	53.26 ± 2.16**
Fucose	157.37 ± 6.85	199.77 ± 4.2**	216.96 ± 12.99**	218.30 ± 11.48**
Sialic acid	45.5 1± 2.57	51.35 ± 1.96	56.35 ± 2.58	95.17 ± 5.54**

Values are mean ± standard error.
Values are expressed as μg/100 mg liver wet weight.
*$P < 0.01$ and **$P < 0.001$ (by ANOVA).

Table 2. Serum levels of glycoprotein moieties in DMN induced hepatic fibrosis

Glycoprotein moiety	Control ($n = 10$)	Day 7 ($n = 10$)	Day 14 ($n = 9$)	Day 21 ($n = 7$)
Hexose[a]	250.04 ± 6.69	184.65 ± 10.08*	128.82 ± 7.92*	149.36 ± 7.23*
Hexosamine[a]	65.08 ± 1.51	63.27 ± 2.29	68.28 ± 2.28	69.54 ± 3.01
Fucose	27.60 ± 1.06	27.02 ± 1.33	29.82 ± 1.36	33.53 ± 1.56*
Sialic acid	26.75 ± 1.09	33.88 ± 1.27*	46.27 ± 1.57*	58.68 ± 2.33*

[a]Protein bound.
Values are mean ± standard error.
Values are expressed as mg/100 ml serum.
*$P < 0.001$ (by ANOVA).

Table 3. Urinary levels of glycoprotein moieties after induction of hepatic fibrosis by DMN

Glycoprotein moiety	Control ($n = 10$)	Day 7 ($n = 10$)	Day 14 ($n = 9$)	Day 21 ($n = 7$)
Total hexose	177.31 ± 18.45	239.79 ± 17.20	431.05 ± 30.08*	658.95 ± 56.99*
Hexosamine	19.98 ± 0.91	23.16 ± 1.55	38.68 ± 2.01*	41.34 ± 1.69*
Fucose	29.21 ± 2.96	28.57 ± 2.46	62.06 ± 3.92*	72.74 ± 7.67*
Sialic acid	16.83 ± 1.23	24.09 ± 1.96	42.81 ± 3.22*	96.82 ± 69*

Values are mean ± standard error.
Values are expressed as mg/100 mg creatinine in 24 hr urine sample.
*$P < 0.001$ (by ANOVA).

increase was striking in comparison with 14th day levels.

Correlation analysis revealed a good positive correlation ($r = 0.969$) between liver and serum hexose levels. However, a negative correlation was observed in the case of liver and serum hexose levels with the urinary excretion of total hexose. Hexosamine and fucose levels in liver and serum indicated 78–95% positive correlation with urinary excretion (Table 4). A good positive correlation was also recorded between liver and serum sialic acid levels ($r = 0.912$) and further with serum sialic acid and its urinary excretion ($r = 0.954$).

DISCUSSION

The results obtained in the present investigation (Tables 1–3) indicate altered metabolism of glycoproteins in the liver following dimethylnitrosamine induced hepatic fibrosis. The increased metabolic turnover of glycoprotein is also reflected in the serum and this is further substantiated by increased urinary excretion. Koizumi *et al.* (1967) reported an increased acid mucopolysaccharide metabolism in chronic hepatic damage caused by serial inhalation of carbon tetrachloride in rats. A characteristic rise in hydroxyproline, hexosamine and galactosamine content were noticed in thioacetamide induced liver fibrosis in rats (Hirayama and Hartmann, 1961). Darcy (1964) observed a rapid increase in serum glycoprotein during necrosis of the rat liver. The appearance of the glycoprotein seemed to be a response to substances liberated from necrotic cells. It was also reported that chronic active liver disease was associated with the accumulation of acid mucopolysaccharides in the perisinusoidal space along with parenchymal cells in areas of necrosis and at the septal parenchymal junction in cirrhotic livers (Galambos, 1966). The acid mucopolysaccharides in human lesions emphasized the responsive role of the hepatic mesenchyme in the pathogenesis of human liver disease (Galambos, 1966).

Many investigators reported that the metabolism of connective tissue proteins is altered during the development of hepatic fibrosis (Bissell *et al.*, 1990; Biagini and Ballardini, 1989; Rauterberg *et al.*, 1981). It has been proposed that connective tissue glycoproteins play an important role in the stabilization of tropocollagen aggregates (Jackson and Bentley, 1968) and in fibril formation and orientation (Robert *et al.*, 1970). The specific recognition of serum glycoprotein carbohydrate chain by reaction with liver cell surface glycoprotein was originally described by Ashwell and Morell (1974) to account for the rapid clearance of glycoproteins with terminal galactose. Poole (1986) reported that proteoglycans play many important roles in the normal physiology of cells and tissues and that disturbances in their metabolism can produce a variety of pathological changes.

The decrease in the content of total hexose in the liver and protein bound hexose in serum indicate an active degradation of glycoprotein moiety in DMN induced hepatic fibrosis. This is also clearly evident from the increased urinary excretion of total hexose, especially on the 14th and 21st days of DMN treatment (Table 3). The decreased hexose moiety could also be because

Table 4. Correlation analysis of liver, serum and urinary levels of various glycoprotein moieties in DMN induced hepatic fibrosis in rats

Combination for correlation	Hexose	Hexosamine	Fucose	Sialic acid
Liver and serum	0.969	0.527	0.665	0.911
Liver and urine	−0.861	0.820	0.776	0.993
Serum and urine	−0.753	0.915	0.946	0.954

Values are correlation coefficients.

none of the major liver glycoproteins, viz. fibronectin, laminin and vitronectin, contains hexose as a major carbohydrate moiety. In our simultaneous study we have noticed an increase in the activity of ß-glucosidase and ß-galactosidase both in liver and serum (unpublished data), which could also support the decreased hexose moiety of glycoprotein in liver and serum. Also, hexoses are associated more directly with collagen rather than glycoproteins, as compared to sialic acid or hexosamine (Sharon, 1975).

The increased levels of hexosamine observed in the liver tissue of rats during hepatic fibrosis concur with an earlier report (Singer and Hutterer, 1959). This suggests an enhanced synthesis of glycoproteins containing hexosamine, especially laminin. A number of investigators have reported increased levels of laminin in the liver during hepatic fibrosis and related liver damage (Hahn *et al.*, 1980; Carlsson *et al.*, 1981; Niemela *et al.*, 1988; Martinez-Hernandez *et al.*, 1991). The increased hexosamine could also be due to the increase in the levels of glycosaminoglycans in hepatic fibrosis (Rauterberg *et al.*, 1981; Koizumi and Nakamura, 1985). Hirayama *et al.* (1961) noticed that an increase in serum galactosamine level correlates with liver fibrosis. But in the present investigation, a normal serum protein-bound hexosamine was observed. This may be due to the increased catabolism of elevated serum protein-bound hexosamines by glycoprotein degrading enzymes. This is supported by the significant increase of hexosamine levels in the urine. An increased level of the major glycoprotein, fibronectin was reported in hepatic fibrosis (Hahn *et al.*, 1980; Pott *et al.*, 1982). It was interesting to note that the glycoprotein moiety fucose was significantly increased in liver on all days of DMN treatment, but not in the serum except on the 21st day. Similarly, sialic acid was significantly increased in serum on all days of DMN treatment but not in the liver except on the 21st day. The explanation of this may be that liver fibronectin has a fucose residue in the core structure as its carbohydrate moiety, whereas plasma fibronectin contains sialic acid residues (Ruoslahti *et al.*, 1981).

The increased urinary excretion of all glycoprotein moieties noticed especially on the 14th and 21st days coincides with the excess mucopolysaccharide derivatives reported in the urine of patients with hepatic fibrosis (Galambos, 1967; Kawata *et al.*, 1959). A number of investigators reported an increased activity of ß-N-acetylglucosaminidase, a glycoprotein degrading enzyme in hepatic fibrosis (Pott *et al.*, 1979, 1982; Ackermann *et al.*, 1981). In our concurrent studies, we have also observed increased activity of glycohydrolases in liver and serum (unpublished data). These findings indicated that in hepatic fibrosis there is an increased degradation of glycoproteins which coincides with the enhanced synthesis.

In hepatic fibrosis, extreme centrilobular necrosis, death and regeneration of liver tissue take place with concomitant influx of inflammatory cells from other sites. So excessive glycoprotein connective tissue in the liver may result either from aggregation of preformed fibers or from formation of new fibers which may be related to the activity of hepatocytes due to necrotic stimulus or the extracellular maturation of fibers and their breakdown. Under inflammatory conditions, it has been shown that not only hydrolytic enzymes are released by the invading cells but also enhanced activities of the enzymes involved in the biosynthesis of glycoproteins, viz. sialyl transferase and galactosyl transferase, in serum and liver of inflamed rats are observed (Fraser *et al.*, 1984). It was reported that the increased glycosylation potential of the liver and the proliferation of liver Golgi complex may play an important role in the secretion of acute phase glycoproteins in turpentine induced inflammation in the rat liver (Lombart *et al.*, 1980). It was also noticed that the ethanol induced impairment of hepatic glycoprotein secretion is mediated by acetaldehyde in inflammation stimulated rats (Volentine *et al.*, 1987). All these findings and the results of the present investigation suggest that not only degradation but also synthesis of glycoproteins takes place at an enhanced rate during hepatic fibrosis. But the balance between synthesis and degradation cannot be maintained under pathological conditions. This results in the excessive accumulation of these matrix components in the space of Disse and sinusoidal walls contributing to the development of portal hypertension, a characteristic feature of hepatic fibrosis which further leads to ascites.

In the normal liver, laminin is absent in the sinusoidal lining. But laminin emerges in the sinusoidal walls during hepatic fibrosis (Hahn *et al.*, 1980; Voss *et al.*, 1980; Jezequel *et al.*, 1990). This coincides with the development of identifiable basement membranes in the space of Disse and decrease in the number of

fenestrations of the endothelial cells (Burt, 1993). The defenestration of endothelial cells leads to increased diffusional barrier and also interferes with the transport of nutrients to hepatocytes contributing to the deterioration of liver functions. All these metabolic alterations are highly significant in the pathophysiology of the development of hepatic fibrosis.

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