CHAPTER II

METABOLISM OF SULPHATED GLYCOSAMINOGLYCANS IN EXPERIMENTAL LIVER FIBROSIS
2.1 INTRODUCTION

Proteoglycans, collagen and structural glycoproteins are the major components of hepatic interstitial connective tissue. Under physiological conditions the total amount of the connective tissue macromolecules comprise only a small fraction of the liver dry weight whereas in response to chronic hepatic injury there is a considerable accumulation of these components in liver (See for review ref. 94, 261, 269, 282).

The metabolism of the macromolecular components of the hepatic connective tissue is particularly significant in view of its tremendous physiological role in providing maximum mechanical stability with minimum hindrance to free transport of fluids, proteins and metabolites between parenchymal cells and blood stream. Proteoglycans are believed to play an important role in meeting these various functions of connective tissue.
The metabolism of proteoglycans in liver has not been studied in detail. The available information on the metabolism of proteoglycans in liver is regarding the glycosaminoglycans. Susuki et al. found that 47% of the total rat liver glycosaminoglycans was HS, 41% was DS and 12% was CS (325). Sampio et al. (292) found that 52% of the total rat liver glycosaminoglycan was HS and 48% DS whereas Nakamura et al. (212) and Ninomiya et al. (222) reported that HS comprises about 80% of the total rat liver glycosaminoglycans. Of the different types of glycosaminoglycans heparan sulphate is the predominant one in normal liver while chondroitin sulphate and dermatan sulphate are present in relatively less amounts. The rate of incorporation of $^{14}$O-glucosamine by perfused rat liver was found to be the largest into HS/H and the smallest for HA (93). Trace amounts of HA and KS have also been reported to be present (93). But there is much variation in the amounts of the glycosaminoglycans particularly DS & CS in liver reported by different workers although similar methods have been used for their identification. In vivo and in vitro studies have shown the synthesis of HS and CS by liver tissue (93, 198, 199) and liver derived cells in culture (222). As discussed in the previous chapter, heparan sulphate proteoglycan has been found to occur at the external surface of hepatocytes (236, 262). Heparan sulphate
proteoglycan has been isolated and purified from rat liver plasma membrane (237). A number of physiological functions such as the control of exchange of metabolites, ions, fluid and water, cell-matrix interaction, accessibility of cell surface receptors etc. have been attributed to heparan sulphate. But not much is known about the physiological function of other types of GAG in liver.

Under pathological conditions such as liver fibrosis, the different types of glycosaminoglycans are found to accumulate in liver. McGee and Patrick (198) concluded from their autoradiographic analysis that mouse liver sinusoidal cells have a capacity for producing glycosaminoglycans which is exaggerated following hepatic injury with CCl$_4$. Further, using $^{35}$S-SO$_4$ incorporation studies they showed that in acute and chronic liver injury with CCl$_4$ there was an increase in the synthesis of CSA and/or C and HS (199). But there was no evidence for the presence of DS or KS in normal or damaged liver. Koizumi found that the amount of acid glycosaminoglycans increased in the liver of rats with chronic liver injury (161). The amount of CSB also increased with the progress of the hepatic damage. This was correlated with an increased level of hyaluronidase which degrades CS A/C but not CS B. Nakamura et al. (211) reported that acid GAG accumulate
in CC\textsubscript{4} induced chronic liver injury due to decreased rate of degradation of these molecules. Koizumi and Nakamura\cite{162} observed an increase in the ratio of CS B to total amount of hepatic acid GAG in carbon tetrachloride - induced liver damage. Suzuki et al. \cite{325} observed that in liver injury produced by CC\textsubscript{4} a low-sulphated DS fraction with a S/hexosamine ratio near unity increased as much as 9 fold on a dry weight basis whereas a high sulphated DS (S/hexosamine 1:3) increased only 2 fold. A significant increase in high sulphated heparan sulphate was also observed by these workers. On the basis of these observations it was suggested that for each of the dermatan sulphate and heparan sulphate class, at least two pools distinguished by their degree of sulphation, turnover rate and physiological function might exist.

Gressner et al \cite{93} studied the synthesis of GAG in slices from normal and acutely injured rat liver with \textsuperscript{14}(C)-glucosamine. The synthesis of GAG was found to increase 80-100\% following acute liver injury. Gressner et al \cite{97} further observed a time dependent biphasic change of total GAG in acute liver injury caused by administration of a high dose of galactosamine hydrochloride. A rapidly occurring inhibition was followed by an enhanced production of \textsuperscript{35}(S)-GAG in later stages of
injury. The synthesis of HS was significantly inhibited in the early stages. The synthesis of CS & DS was more stimulated than that of heparan sulphate in the later stages of injury.

Stuhlsatz et al (323) analysed the different GAG in normal and cirrhotic human liver. The levels of total as well as individual GAG increased significantly in liver of patients with alcoholic cirrhosis, the increase in DS & CS being more pronounced than that of HS. In a recent report the acid GAG of human cirrhotic liver was found to be 5-6 times more than that in the normal liver (210). The increase in HS & DS reflected an increase in total GAG with advancing liver cirrhosis. The ratio of the unsulphated to sulphated GAG was found to be increased at the early stages of cirrhosis and decreased to near normal values with the progress in fibrotic process.

Apart from studying the rate of synthesis of glycosaminoglycans by liver in vivo and in vitro using $^{35}$S-$SO_4$ or $^{14}$C-glucosamine, some attempts have also been made to study the enzymes concerned with the degradation of GAG. Koizumi (161) reported an increase in the activities of hyaluronidase in $CC_4$ injured rat liver. Weber et al (361) found an increase in the activity of /N-acetyl-D-glucosaminidase, /D-glucuronidase, /L-iduronidase and cathepsin in non parenchymal cells during thioacetamide
induced chronic liver damage; but the activity of hyaluronidase was found to be reduced by 40-50%. The activity of aryl sulphatase showed a decrease in the early stages and reached almost control levels in the later stages. Parenchymal cells contain strongly elevated levels of these enzymes except hyaluronidase and α-L-iduronidase. Hyaluronidase showed decrease/no significant change in thioacetamide induced damage in liver in these studies. GAG degrading lysosomal enzyme activities in reversible stages of thioacetamide fibrosis were found to decrease in other studies (131, 160). Thus it appears that depending on the type of toxic agent that induces the liver damage and the developmental stage of fibrosis there is change in the activity of individual lysosomal hydrolases.

Akihisa isolated glycosaminoglycans from the liver of rats of different ages (128a). The amount of total GAG was found to increase at 3-7 weeks, remained at plateau level at 7-21 weeks and slightly decreased at 21-35 weeks. In all ages HS was found to be the major component with DS, CS & HA being minor components. The amount of HS & DS increased whereas CS decreased at 3-7 weeks.

The above reports show an accumulation of GAG in liver in fibrosis. An increased incorporation of radioactive sulphate into polysaccharide, accumulation of DS
in large amounts, shift in the relative amounts of the different types of GAG and even qualitative changes in these glycosaminoglycans have been reported in experimental liver fibrosis. There are some reports which indicate accumulation of HS & CS whereas other reports show an accumulation of DS. During acute and chronic experimental hepatic injury an increase in the rate of synthesis of HS and CS has also been observed. However adequate experimental data is not available to explain how the metabolism of GAG is affected leading to accumulation of GAG in liver under these pathological conditions. Therefore detailed investigations have been undertaken in carbon tetrachloride treated rats, an experimental model for studies on liver fibrosis (133a, 269a, 269b).

2.2 MATERIALS AND METHODS

2.2.1 Treatment of rats:

Male sprague Dawley rats weighing 150-180g fed Hindustan Léver rat diet were used for these studies. The animals were divided into control and experimental groups. Experimental animals received 0.1 ml/100g body weight of carbon tetrachloride in ground nut oil (1:1 v/v) through an intra gastric tube every 72 hrs for 2 months and control animals received the same amount of ground nut oil during
this period. The rats were killed at the end of the experimental period; livers were taken into ice cold containers.

2.2.2 Analysis of Glycosaminoglycans.

The total glycosaminoglycans were isolated from dry defatted tissue. The individual glycosaminoglycans were separated and quantitated. The details of the procedure are given in chapter VI.

2.2.3 Kinetics of $^{35}$S-Sulphate incorporation.

Fresh liver slices of 0.5 mm thickness were incubated in 5-7 ml minimum essential medium in presence of 5/μCi/ml radio active sodium sulphate in small Erlenmeyer flask for different time intervals at 37°C in a shaker water bath. At the end of each incubation, the slices were transferred, washed in cold Krebs-Ringer buffer containing 0.005 M sodium sulphate and defatted with acetone in the cold. The dried acetone washed pellet was digested with papain and the $^{35}$S-GAG was collected as described in Chapter VI.

The rate of degradation of sulphated GAG in liver was studied in vitro by following the decrease in the content of $^{35}$S-GAG in liver slices, prepared from animals injected with 0.5 mCi $^{35}$S-sodium sulphate 2 hrs before
the experiment. The liver slices were incubated for different time intervals in minimum essential medium containing 0.5 mM sodium sulphate at 37°C as above. At the end of each incubation period the slices were washed and the remaining amount of $^{35}(S)$-GAG in tissue was determined as described above.

2.2.4 Assay of enzymes

The activity of D-glucosamine 6-phosphate isomerase (glutamine forming) (EC 5.3.1.19) was determined in liver homogenate by the procedure of Pogell and Gryder (253) and that of UDPG dehydrogenase (EC 1.1.1.22) by the procedure of Strominger et al. (320). The activity of β-N-acetyl hexosaminidase (EC 3.2.1.30) was determined using p-nitrophenoxyβ-D-glucosaminide as substrate (141), β-glucuronidase (EC 3.2.1.31) using p-nitrophenoxyβ-D-glucuronide (141) and aryl sulphatase (EC 3.1.6.1) using nitro catechol sulphate (289). Serum glutamate pyruvate transaminase (EC 2.6.1.2) was determined by the method of Reitman and Frankell (270). The details of the procedure are given in Chapter VI.

2.2.5 Other methods.

Liver triglycerides were determined by the method of Van Handel and Zilversmit (351). The amount of collagen
in liver was estimated as hydroxy proline by the method of Neuman and Logan (220) after hydrolysis with 6N HCl for 6 hrs at 120°C in sealed tubes. Protein was estimated by the method of Lowry et al. (191). The details of the procedure are given in Chapter VI.

2.3 RESULTS

2.3.1 Production of Liver Injury

Treatment with CCl₄ resulted in liver injury as evidenced by enhanced levels of serum GPT (Table 3). Steatosis in liver was indicated by elevated levels of triglycerides and fibrosis was indicated by elevated levels of hydroxy proline in CCl₄ treated rats as compared with untreated rats. The diet consumption of CCl₄ treated rats (12.5 ± 1.6) was similar to that of the control animals (13.3 ± 1.5). The average gain in weight (g) at the end of the experiment was 60 ± 7 for CCl₄ treated rats and 69 ± 8 for untreated rats.

2.3.2 Concentration of glycosaminoglycans in liver.

The results are given in table 4. Heparan sulphate was the predominant glycosaminoglycan in normal liver comprising about 75% of the total GAG, the remaining being dermatan sulphate and chondroitin sulphate A/C. There was an increase in the total GAG in CCl₄ treated rats. The individual glycosaminoglycans also increased though not in
Table 3. Levels of Triglycerides and Hydroxy proline in liver and glutamate: pyruvate transaminase Activity in serum

<table>
<thead>
<tr>
<th>Group</th>
<th>Triglycerides (mg gly/100 g tissue)</th>
<th>Hydroxy-proline (mg/100g liver)</th>
<th>sGPT (units/litre)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated control</td>
<td>305 ± 15</td>
<td>13.9 ± 1.5</td>
<td>52 ± 3.5</td>
</tr>
<tr>
<td>CCl₄ treated</td>
<td>830 ± 37</td>
<td>49.5 ± 5.0</td>
<td>410 ± 15.0</td>
</tr>
</tbody>
</table>

Average of the values from 6 - 8 animals ± SE p < 0.01

Average wet weight of liver of rats in group 1 was 6.1 ± 1.0 and that in group 2 was 7.4 ± 1.2
Table 4. Concentration of different Glycosaminoglycans in liver (µg uronic acid/g dry tissue \( \bar{x} \pm SE \))

<table>
<thead>
<tr>
<th>Group</th>
<th>Total</th>
<th>HS</th>
<th>CS</th>
<th>DS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated control</td>
<td>65 ± 5.2</td>
<td>48 ± 4.1</td>
<td>4.9 ± 0.7</td>
<td>6.4 ± 0.5</td>
</tr>
<tr>
<td>CCl4 treated</td>
<td>170 ± 11.4</td>
<td>117 ± 8.0</td>
<td>13.5 ± 1.3</td>
<td>29.0 ± 2.5</td>
</tr>
</tbody>
</table>

Average of the values from six rats ± SE. p < 0.01
a parallel manner. There was about 2.5 fold increase in HS whereas DS was about 4 times and chondroitin sulphate was about 3 times higher in the \textsubscript{CCl}_4 treated animals. These results confirmed that the \textsubscript{CCl}_4 induced liver fibrosis is associated with an accumulation of GAG in liver.

2.3.3 Sulphate Kinetics

In order to study how the metabolism of GAG is affected in \textsubscript{CCl}_4 induced liver fibrosis, the rate of incorporation of \textsuperscript{35}S-S\textsubscript{O}_4 into total GAG was studied in vitro \textit{in vivo} in liver slices (Fig.3). The incorporation of radioactivity was almost linear upto 6 hrs in slices prepared from untreated rat liver whereas in \textsubscript{CCl}_4 treated rats it was linear for 4-6 hrs. The rate of \textsuperscript{35}S-S\textsubscript{O}_4 incorporation into total GAG was significantly higher in liver slices prepared from \textsubscript{CCl}_4 treated rats than that of the untreated controls, probably indicating that the rate of synthesis is higher in \textsubscript{CCl}_4 induced liver fibrosis.

2.3.4 Activity of D-glucosamine-6-phosphate isomerase (glutamine forming) and UDPG dehydrogenase.

D-glucosamine-6-phosphate isomerase (glutamine forming) (EC 5.3.1.19) and UDPG dehydrogenase (EC 1.1.1.22) are two key enzymes involved in the formation of hexosamine and UDP glucuronic acid precursors respectively in the
Legends to Figure

Fig. 3. **Incorporation of $^{35}(S)$-SO$_4$ into total Liver glycosaminoglycans**

Slices were prepared from the livers of rats in which chronic liver injury was induced by the administration of CCl$_4$ and untreated normal control. The slices were incubated in medium containing $^{35}(S)$-Na$_2$SO$_4$ at 37°C for different time intervals as described in methods. The total $^{35}(S)$-GAG were isolated at the end of each incubation period and the radioactivity was measured. The values given are the means and the ranges of triplicate analysis. (●) untreated control (○) CCl$_4$ treated.
Fig. 3
biosynthesis of GAG. In fibrotic liver the activity of
D-glucosamine-6-phosphate isomerase was significantly higher
than that of the control. Similarly the activity of UDPG
dehydrogenase was also higher in CCl₄ treated rat liver
(Fig.4). The enhanced activity of these enzymes can lead
to an increase in the availability of the precursors for
the biosynthesis of GAG.

2.3.5 Activity of β-glucuronidase (EC 3.2.1.31),
β-Hexosaminidase (EC 3.2.1.32) and aryl sulphatase
(EC 3.2.6.1).

β-glucuronidase and β-N-acetyl glucosaminidase are
both exoenzymes splitting off uronic acid and hexosamine
residues respectively from one end of the polysaccharide
chain. Sulfate moieties are cleaved off by sulphatases
(See Introduction for details).

There was no significant difference in the activity
of β-glucuronidase and β-hexosaminidase in liver between
animals treated with CCl₄ and control (Fig.4). The activity
of aryl sulphatase (though not significant) was less than the
untreated controls (Fig.4).

2.3.6 Degradation of ³⁵(S)-polysaccharides

The results of in vitro chase of the pulse labelled
polysaccharides are given in fig.5. At different periods of
Fig. 4. Activity of some of the enzymes concerned with the metabolism of GAG.

The average specific activities of different enzymes in liver of COCl₂ treated animals (□) are expressed as percentage of the untreated control (□) a) glucosamine-6-phosphate isomerase (glutamine forming (23 ± 1.2 μ moles hexosamine/hr/mg protein); b) UDPG-dehydrogenase (85 ± 1.1 units/mg protein; c) β-glucuronidase (92 ± 1.6 μg p-nitrophenol/hr/mg protein); d) β-hexosaminidase (30 ± 0.7 μg p. nitrophenol/hr/mg protein) and (e) Aryl sulphatase (40±1.5 μg nitrocatechol/hr/mg protein). The values given in parenthesis are the average specific activity of each enzyme ± SE (n=6) in untreated control rat liver.
Fig. 4

- Control
- Experimental

Percentage of activity
Fig. 5. Degradation of \( ^{35}(S) \)-GAG in vitro

Livers from animals injected \( ^{35}(S) \)-sulphate 2 hrs before killing them were sliced, washed in cold medium and incubated for varying time intervals at 37°C. The amount of \( ^{35}(S) \)-GAG remaining in liver slices after each period of chase is expressed as percentage of the initial amount.

(●) Untreated normal control (Ο) CCl\(_4\)-treated.
Fig. 5
chase under identical conditions the fraction of $^{35}(S)$-GAG remaining in the tissue from CCl$_4$ treated rats was higher than that of the untreated controls indicating a difference in the rate of degradation. The half life of $^{35}(S)$-GAG was 24-25 hrs in CCl$_4$ treated rat liver slices whereas in normal control rats it was 8-9 hrs. The difference in the rate of degradation might not be due to difference in the reutilization of free radioactive sulphate formed during the degradation as its specific activity would be considerably reduced due to the presence of added sodium sulphate in the medium.

2.4 DISCUSSION

The amount of acid glycosaminoglycans has been found to be much less in rat liver than in other tissues such as aorta and skin which are rich in connective tissue. Heparan sulphate is the predominant GAG comprising about 75% of the total GAG. Dermatan sulphate comprises about 10% of the total GAG whereas chondroitin sulphate A & C together forms about 8% of the total GAG. Previous reports (121) have shown that total amount of glycosaminoglycan in rat liver is low though the actual amount reported varied from 0.1 to 0.35 mg. (for review see 171). Different reports show considerable variation in the relative amounts of individual
glycosaminoglycans. However heparan sulphate has been shown to be the predominant GAG in all these cases.

The chronic hepatocellular damage with CCl\textsubscript{4} has resulted in the accumulation of connective tissue protein such as collagen in rat liver. This has also resulted in the accumulation of proteoglycans as evidenced by increased levels of their polysaccharide chains. The increase in the individual glycosaminoglycans did not proceed in a parallel manner. The DS was found to accumulate to a greater extent when compared with HS & CS. There was about 4 fold increase in DS whereas HS showed about 2 - 2.5 fold increase. Similar results have been reported in human liver cirrhosis (210, 323) and drug induced chronic liver fibrosis in rats (161, 162, 211, 325) and mouse (198, 199), though the extent of accumulation of each GAG observed in each case was different. McGee and Patrick (199) did not find any accumulation of DS whereas Koizumi et al. (162) found an increase in DS in chronic hepatic injury in rats. Dermatan sulphate and chondroitin sulphate are shown to be prominent in the fibrotic liver than normal (76). There is a general agreement regarding the accumulation of DS: the extent of accumulation of the GAG may depend on the nature and duration of the liver injury.

The increase in total GAG as well as individual GAG can be either due to an increase in the rate of their
synthesis or by a decreased rate of their degradation or due to both. Their accumulation was generally believed to involve a stimulated synthesis rather than reduced degradation. This conclusion was reached mostly based on $^{35}(S)-\text{SO}_4$ or $^{14}(C)$-glucosamine incorporation studies. Our results show that one of the reasons for the accumulation of glycosaminoglycans is increased rate of synthesis as evidenced by increased rate of $^{35}(S)-\text{SO}_4$ incorporation and increase in the activity of biosynthetic enzymes such as glucosamine 6-phosphate isomerase and UDPG dehydrogenase. The rate of $^{35}(S)-\text{SO}_4$ incorporation probably equals the rate of carbohydrate chain synthesis. It has not been shown so far that sulphate groups of liver GAG turnover independently from the carbohydrate backbone. Further, Gressner, et al. found that there was no significant change in the specific activity of $^{35}(S)$-PAPS in injured liver (98). D-glucosamine-6-phosphate isomerase (glutamine forming) and UDPG dehydrogenase are two key enzymes involved in the formation of hexosamine and uronic acid precursors for the biosynthesis of GAG. D-glucosamine-6-phosphate isomerase (glutamine forming) catalyses one of the rate limiting steps in the biosynthetic pathway of GAG, its activity being inhibited by UDP-N-acetyl glucosamine (163). UDPG dehydrogenase is involved in the formation of UDP-glucuronic acid and UDP-iduronic acid and is subject to inhibition by UDP-xylose(218).
Increased activity of glucosamine-6-phosphate isomerase and UDPG-dehydrogenase can lead to increased availability of UDP-hexosamine and UDP-glucuronic acid precursors which may result in increased biosynthesis of GAG in liver.

An alteration in the rate of catabolism of proteoglycans can also result in the accumulation of proteoglycan in liver under fibrotic condition. The results of pulse-chase studies show that 35(S)-proteoglycans in fibrotic liver have a longer half life than that in untreated liver. The kinetics of degradation in vivo may not be identical to that of in vitro conditions. However a longer half life observed under in vitro conditions may be one of the reasons for the accumulation of GAG in fibrotic liver. But the activity of some of the enzymes concerned with the degradation of GAG namely β-glucuronidase β-hexosaminidase and aryl sulphatase determined using synthetic substrates were not significantly different. Weber et al (361) found an increase in the activity of all these enzymes except hyaluronidase, which was reduced in non parenchymal cells and remained unaffected in parenchymal cells and aryl sulphatase, which was reduced in early stages and reached normal levels in later stages of thioacetamide induced liver fibrosis. Hutterer observed a decrease in (131) GAG degrading lysosomal hydrolase activity in the reversible stage of thioacetamide fibrosis. But Koizumi (161) had
reported an increase in hyaluronidase in CCl₄ induced liver fibrosis. Our studies did not show any significant difference in the activity of any of these enzymes though there is a tendency for β-hexosaminidase to increase and aryl sulphatase to decrease probably because of the difference in the degree of fibrosis induced with CCl₄ under the conditions of the experiment. The activities of other lysosomal hydrolases have not been studied. If the extracellular ³⁵(S)-proteoglycans/GAG are taken up into the lysosomes at a different rate in fibrotic liver, the rate of degradation can also be impaired inspite of similar activities of lysosomal enzymes. Further, unequal distribution of different proteoglycans, particularly into the extracellular matrix in normal and fibrotic liver and a difference in the rate of degradation of the extracellular proteoglycan, particularly dermatan sulphate, can probably result in a slower degradation of the proteoglycan in fibrotic liver. The possibility of an alteration of intralysosomal pH as a result of any change in ATP levels under fibrotic conditions, resulting in a decreased lysosomal degradation of GAG cannot also be excluded. Probably such a reduced rate of catabolism of sulphated GAG in vivo, can be one of the factors contributing to the accumulation of proteoglycans in fibrotic liver. Further studies are required to understand the mechanism of alteration in the catabolic rate of GAG in liver fibrosis.
The proliferation of liver cells in response to hepatic injury may be related with the changes in the metabolism of proteoglycans. But the nature and origin of those cells which are responsible for enhanced synthesis of connective tissue components during liver fibrosis are not fully understood. Primary cultures of rat hepatocytes have been shown to synthesise almost exclusively heparan sulphate in a protein bound form (236, 262). But it is not known which type of cells produce CS & DS. The cellular source of chondroitin sulphate and dermatan sulphate synthesis needs to be clarified to understand the molecular mechanism of the disease-associated changes in proteoglycan metabolism. An extra hepatic origin of CS & DS is less likely as the liver tissue can incorporate $^{35}$S-$\text{SO}_4$ and $^{14}$C glucosamine in vitro into nitrous acid resistant and chondroitinase susceptible polysaccharides (93, 98). The biosynthetic capacity of the non parenchymal liver cells and of infiltrated inflammatory cells has to be analysed to study the source of CS & DS. It has been proposed that fat-storing Ito cells are probable candidates which proliferate under injury (171). Based on electronmicroscopic studies fat storing Ito cells have been suggested to develop to fibroblast-like connective tissue synthesising cells after experimental liver injury (145). It is also not known what factors control the hepatic cell proliferation even though
different reports claim the presence of hormonal and nonhormonal factors responsible for inducing proliferation (66, 89, 202, 214). Our previous observations indicated that Bt₂ cAMP and hormones which produce cAMP reduced the synthesis of HS by primary cultures of rat hepatocytes (324). Further, the presence of fetal calf serum has been found to reduce the synthesis of sulphated GAG by hepatocytes indicating the presence of some factors in serum controlling the synthesis of hepatic proteoglycans.
The metabolism of glycosaminoglycans (GAG) in liver of rats subjected to hepatic injury with carbon tetrachloride has been studied. The treatment with carbon tetrachloride resulted in a 2.5 fold increase in total GAG in liver. The individual glycosaminoglycan followed a different pattern, heparan sulphate being 2.5 times and dermatan sulphate over 4 times higher than control. The rate of \((^{35}\text{S})\)-sulphate incorporation into GAG was significantly higher in liver slices prepared from CC1\textsubscript{4} treated rats than the control. Further, the activities of aminotransferase and UDPG dehydrogenase, two key enzymes of GAG biosynthetic pathway, were also high suggesting an enhanced rate of synthesis of GAG in CC1\textsubscript{4} induced liver injury. The \((^{35}\text{S})\) GAG were degraded under in vitro conditions with a half-life of 8-9 hrs in untreated liver whereas those in CC1\textsubscript{4} treated animals were degraded with a half-life of 24-25 hrs, indicating a reduced rate of degradation of GAG in CC1\textsubscript{4} induced hepatic injury. But the activities of some of the lysosomal enzymes involved in the degradation of GAG such as \(\beta\)-glucuronidase, \(\beta\)-hexosaminidase and aryl sulphatase were not significantly different. These results indicate that in CC1\textsubscript{4} induced hepatic injury the glycosaminoglycans accumulate in liver probably as a result of an enhanced rate of synthesis coupled with reduced rate of degradation.