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Degradation of [^{3}H]Chondroitin 4-Sulphate and Re-utilization of the [^{3}H]Hexosamine Component by the Isolated Perfused Rat Liver

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Radiolabelled chondroitin 4-sulphate was isolated after incubation of rat rib cartilage with *N*-acetyl-D-[6-^{3}H]galactosamine. After proteolytic digestion of the tissue with either papain or trypsin the released [^{3}H]chondroitin 4-sulphate was added to an isolated perfused rat liver system. Analysis of perfusate after several hours perfusion showed that radiolabelled amino sugars were secreted by the liver in a low-molecular-weight form and as components of glycoproteins.

In the rat, the liver has been identified as the major site of accumulation of radioactivity after intravenous administration of chondroitin 4-[^{35}S]-sulphate (Wood *et al.*, 1973*a*). Degradation of this polymer with the release of inorganic [^{35}S]sulphate into the plasma has been demonstrated in the whole animal (Wood *et al.*, 1973*a*) and in the isolated perfused rat liver (Wood *et al.*, 1973*b*). Oligosaccharide [^{35}S]sulphates have been identified as intermediates in the liver (Wood *et al.*, 1973*a*, 1976). However, these do not appear in the plasma, but are further metabolized to inorganic [^{35}S]sulphate, before release of radioactivity into the circulation.

The fate of the carbohydrate components of glycosaminoglycans after desulphation is not known, although there are indications that mechanisms exist for their re-utilization. For example, in whole-animal studies, after intravenous administration of chondroitin 4-sulphate radiolabelled with ^{3}H in the hexosamine residues with ^{35}S in the sulphate group, the amount of ^{35}S excreted in the urine (expressed as a percentage of the injected dose) was far greater than the amount of ^{3}H excreted (Wood *et al.*, 1976). Analysis of rat liver for radioactive components after intraportal injection (McGarrahan & Maley, 1962) or perfusion (DelGiacco & Maley, 1964) of D-[1-^{14}C]glucosamine demonstrated that a pathway exists in liver for the synthesis of UDP-*N*-acetylhexosamines and of sialic acid from free hexosamines. An analogous pathway was identified for the utilization of *N*-acetylgalactosamine (Maley *et al.*, 1968).

In the rat, intraperitoneal administration of D-[1-^{14}C]galactosamine results in the incorporation of radioactivity into serum proteins with the greatest concentration in the α-globulins (White *et al.*, 1965). The serum proteins taken 6h after injection were hydrolysed and the hexosamines separated by paper chromatography; glucosamine had a ^{14}C content over ten times that of galactosamine.

Previous studies in this laboratory (MacNicoll *et al.*, 1977, 1978) have shown that radiolabelled *N*-acetyl-D-galactosamine is also incorporated by the isolated perfused rat liver into the carbohydrate component of glycoproteins, which are subsequently secreted into the plasma. Radiolabel was incorporated into a number of different glycoproteins predominantly in the form of sialic acid and *N*-acetylglucosamine residues. Deacetylation was found not to be a prerequisite for incorporation of the *N*-acetylhexosamine into glycoproteins.

The present study was undertaken to investigate the possibility that *N*-acetylgalactosamine released during hepatic degradation of polymeric chondroitin 4-sulphate is reutilized in a manner similar to that described for monomeric hexosamines, i.e. to demonstrate that the components of one polymer can be recycled and used for the synthesis of other polymers.

Materials and Methods

The materials and the experimental techniques used were as described by MacNicoll *et al.* (1978) except for the following.

Radiolabelling and isolation of chondroitin 4-sulphate from rat rib cartilage

The cartilaginous medial region of the ribs, from 8-week-old male and female M.R.C. hooded rats, was dissected free from surrounding tissue and

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sliced into segments approx. 1 mm in length. These segments (5–14 g wet wt.) were suspended in 10–20 ml of Krebs–Ringer bicarbonate buffer, pH 7.3 (Umbreit *et al.*, 1964). D-[6-^{3}H]Glucosamine (1 mCi, 19.6 Ci/mmol: The Radiochemical Centre, Amersham, Bucks., U.K.) was added and the mixture was incubated for 3 h at 37°C. The supernatant was then removed by decanting, and the residue was washed with a further volume (10–12 ml) of Krebs–Ringer bicarbonate buffer. The insoluble residues were subjected to proteolytic digestion with either papain or trypsin.

Digestion with papain

The residues were suspended in 0.1 M-sodium acetate, 10 mM-L-cysteine, pH 5.5 (10–20 ml) and incubated for 16 h at 60°C with papain (EC 3.4.22.2) from papaya juice (5 mg; 4 units/mg; twice crystallized; BDH Chemicals, Poole, Dorset, U.K.). A second addition of papain (5 mg) was made and incubation was carried out for a further 8 h at the same temperature.

Digestion with trypsin

The residues were suspended in 0.05 M-Tris/HCl/0.002 M-$CaCl_2$ (pH 8.3) (10–20 ml) and incubated with pancreatic trypsin (400 mg; 0.5 Anson units/mg; EC 3.4.21.4; BDH Chemicals) for 16 h at 37°C. A second addition of enzyme (400 mg) was made and incubation continued at 37°C for a further 8 h.

All digests were subsequently treated in the same way. Each digest was dialysed (16 h at 4°C) against 0.15 M-NaCl (2 × 2 litres) and residual protein was removed on a mixed bed of Amberlite resin (IRC-50, IR-120; 1:1, w/w; H^+ forms; BDH Chemicals). The mixed Amberlite bed (200 mm × 20 mm) was prepared by washing with 2 M-HCl (250 ml), followed by water until the effluent returned to pH 7.0. The non-diffusible material remaining after dialysis was applied to the column and eluted with water. The pH of the effluent was monitored continuously, and when the pH fell below neutral, the effluent was collected. Collection was stopped when the pH returned to neutral. The acidic effluent (70–100 ml) was neutralized with 2 M-NaOH and adjusted to 1 M with respect to NaCl. Chondroitin 4-sulphate was isolated from the neutralized effluent by chromatography on columns (100 mm × 9 mm) of Dowex 1 (X2; Cl^- form; 200–400 mesh; Sigma Chemical Co., St. Louis, MO, U.S.A.) as described by Mason & Wusteman (1970).

Labelled chondroitin 4-sulphate preparations were assayed for glucuronic acid (Bitter & Muir, 1962) and hexosamine content (by amino acid analyser; MacNicoll *et al.*, 1978) and any low-molecular-weight contaminants sought by molecular-exclusion chromatography. The polymer samples isolated by trypsin digestion gave single peaks of radioactivity close to the void volume of columns of Sephacryl S-200 and were used without further fractionation. The sample obtained by papain digestion contained a wider range of chain lengths so those of larger hydrodynamic volume were selected by collecting the fraction eluted between 13 and 23 ml from a column (590 mm × 9 mm) of Sephadex G-200 eluted with 0.5 M-NaCl at a flow rate of 1 ml/h (void volume 13 ml).

Isolated-liver perfusion

The method used was that of Curtis *et al.* (1970), and perfusion was carried out at a flow rate of 20–67 ml/min for 5–8 h with homologous heparinized rat blood containing [^{3}H]chondroitin 4-sulphate (4.1–9.0 μmol). Samples of the perfusate (1 ml) were collected every hour. At the end of each perfusion the entire perfusate was collected and the plasma separated by centrifuging (3000 *g* for 30 min at 4°C). The liver was washed free of blood with 0.15 M-NaCl (10 ml) administered via the portal vein. The washed liver was homogenized in 0.15 M-NaCl (15 ml) in a Potter–Elvehjem homogenizer (0.23 mm clearance).

Incubation of [^{3}H]chondroitin 4-sulphate with homologous heparinized blood

In control experiments, each preparation of [^{3}H]-chondroitin 4-sulphate was incubated with homologous heparinized blood for 5–8 h at 37°C and aerated with O_2/CO_2 (19:1). The concentrations of each glycosaminoglycan preparation corresponded to those used in the isolated rat liver system. Samples were removed every hour and the plasma separated by centrifuging (3000 *g* for 30 min at 4°C).

Molecular-exclusion chromatography

Plasma samples (100–500 μl) or samples of [^{3}H]-chondroitin 4-sulphate were subjected to chromatography on Sephadex G-200 (MacNicoll *et al.*, 1978) or alternatively on columns (580 mm × 9 mm) of Sephacryl S-200 (Pharmacia, London, U.K.). The preswollen gel was equilibrated with 0.15 M-NaCl/8 M-urea; samples were applied to the column and eluted with the same solvent at a flow rate of 3 ml/h. Effluent fractions (1 ml) were collected and assayed for radioactivity by liquid-scintillation counting.

Ion-exchange chromatography

Plasma samples (1–10 ml either dialysed against water or non-dialysed) were adjusted to 0.15 M-NaCl/8 M-urea and applied to a DE-52 DEAE-cellulose column (110 mm × 9 mm; Whatman Biochemicals, Maidstone, Kent, U.K.) equilibrated

with the same solvent. A linear salt gradient, either 0.15–0.6M-NaCl or 0.15–2.0M-NaCl in urea, was applied to the column at a flow rate of 4ml/h. Effluent fractions (2ml) were collected and radioactive contents of samples (50–500μl) were determined by scintillation counting.

Experimental and Results

The metabolism of [^{3}H]chondroitin 4-sulphate preparations by the isolated perfused rat liver

Four separate preparations of [^{3}H]chondroitin 4-sulphate were used in the present study, all isolated from rat rib cartilage that had been labelled by incubation with D-[6-^{3}H]glucosamine (Table 1). One (I) was isolated by papain digestion of the whole cartilage and was used after removal of fractions of low molecular weight by gel chromatography. The other three (II, III and IV) were obtained by using the milder proteolytic agent trypsin to remove the more readily accessible regions of the polypeptide chain yielding chondroitin sulphate chains with appreciable quantities of covalently bound peptide (Wood *et al.*, 1976).

As a result of varying enzyme/tissue ratios and incubation conditions during labelling [e.g. whether the cartilage was flushed with O_2/CO_2 (19:1) before addition of the glucosamine] the trypsin-digested preparations exhibit various specific activities, different elution profiles on gel chromatography and (from the glucosamine content) contain minor quantities of covalently attached keratan sulphate.

In separate experiments each preparation was added to an isolated perfused rat liver system. The amount of [^{3}H]chondroitin 4-sulphate added in each case and the details of each perfusion are summarized in Table 2. At the end of each perfusion, the distribution of radioactivity in the plasma between the glycoprotein, chondroitin 4-sulphate and low-molecular-weight fractions and the amount of radioactivity remaining in the corresponding livers was determined.

Gel chromatography of plasma samples after perfusion with labelled chondroitin sulphate

Chromatography on Sephadex G-200 of plasma after perfusion with preparation I (500μl) showed a progressive increase in the amount of radioactivity eluted by an effluent volume corresponding to the totally included volume of the column (Fig. 1). This radiolabelled low-molecular-weight component released by the liver accounted for 9.1, 18.1 and 22.6% of the total radioactivity in plasma after 2, 4 and 5h perfusion respectively. Similar results were obtained with preparations II (6.5% in the low-molecular-weight components after 5h perfusion) and III (21.3% after 8h), whereas preparation IV yielded no radioactive components of low molecular weight after 8h perfusion.

Table 1. *Analysis of [^{3}H]chondroitin 4-sulphate preparations*

	Hexosamine/uronic acid ratio (mol/mol)	Galactosamine/glucosamine ratio (mol/mol)	Radioactivity as glucosamine (% of total)	Radioactivity as galactosamine (% of total)	Specific radioactivity (μCi/mmol of hexosamine)	Elution volume of peak of radioactivity from Sephacryl S-200 columns (ml)
Preparation after digestion with papain (I)	1.06	*	*	>99.9	258	20
Preparations after digestion with trypsin:						
II	1.05	32.9	2.2	97.8	401	19
III	1.05	52.1	1.3	98.7	573	17
IV	1.05	*	1.8	98.2	645	22

* Glucosamine not detected by amino acid analyser.

Table 2. *Details of isolated rat liver perfusions for studying the metabolism of [^{3}H]chondroitin 4-sulphate*

Abbreviation: N.D., not determined.

Perfusion with preparation:	Administered dose of chondroitin 4-sulphate (μCi)	Administered dose of chondroitin 4-sulphate (μmol of hexosamine)	Duration of perfusion (h)	Liver weight (g)	Initial blood volume (ml)	Haematocrit (% cells)	Perfusion rate (ml/min)	^{3}H radioactivity recovered in bile (% of administered dose)	^{3}H radioactivity recovered in liver after perfusion (% of administered dose)
I	1.2	4.7	5	9.9	110	34–36	20–40	N.D.	19.0
II	1.65	4.1	5	11.7	110	30–33	22–32	0.04	15.7
III	4.4	7.7	8	13.0	110	30–36	35–67	0.02	5.6
IV	5.8	9.0	8	14.5	110	31–36	31–49	0.21	10.7

Fig. 1. *Chromatography on Sephadex G-200 of [^{3}H]-chondroitin 4-sulphate and its metabolites in the plasma after isolated-liver perfusion*

Elution profiles show the distribution of radioactivity in (*a*) chondroitin sulphate preparation I, and in plasma obtained after (*b*) 2h and (*c*) 5h liver perfusion. The columns were eluted with 0.2M-NaCl, the void volume is indicated by V_0 and the totally included volume by V_t.

Examination of the diffusible and non-diffusible fractions of plasma for radioactive components

To obtain enough of the component(s) of low molecular weight for hexosamine analysis plasma obtained after 8h perfusion with preparation III (10ml) was dialysed against water (50ml) for 16h at 4°C. The diffusible material, which accounted for 22% of the radioactivity in plasma, was freeze-dried and redissolved in water (4ml). A sample (1ml) of this material was hydrolysed with acid and analysis showed that 91.5% of the radioactivity in the diffusible fraction was present as hexosamines, 56.3% as [^{3}H]galactosamine and 35.2% as [^{3}H]-glucosamine.

Ethanol fractionation (Cohn *et al.*, 1950) of the non-diffusible fraction of plasma (10ml) showed (Table 3) that the majority (68.4%) of the radioactivity precipitated in Cohn fractions I,III-3. This represented 53.5% of the total radioactivity in plasma obtained after 8h perfusion. Radioactivity associated with Cohn fractions VI and V accounted for 13.3 and 6.6% respectively of the total radioactivity in plasma after 8h perfusion.

Table 3. *Ethanol fractionation of the non-diffusible fraction of plasma obtained after 8 h liver perfusion with [^{3}H]chondroitin 4-sulphate (preparation III)*

For details see the text.

Cohn fraction no.	Major components	Radioactivity (% of total in all fractions)
II	γ-Globulins	1.8
III-0	β-Globulins	2.0
III-1, 2	Prothrombin isoagglutinins	1.2
I,III-3	Fibrinogen cryoglobulins	68.4
VI	Acid glycoproteins	17.0
V	Albumins	8.4
IV-6, 7	α-Globulins	1.4
IV-1	Glycolipids	—

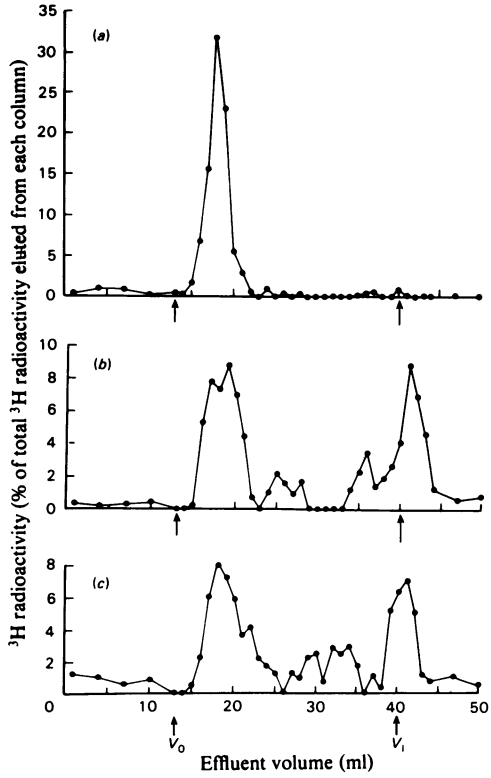

Fig. 2. *Chromatography on Sephacryl S-200 of neuraminidase-digested plasma fractions obtained after 8 h liver perfusion with [^{3}H]chondroitin 4-sulphate (preparation II)*

The non-diffusible fraction of plasma was subjected to ethanol fractionation. Fractions were digested with neuraminidase, and the elution profiles show the distribution of radioactivity after digestion of (*a*) Cohn fraction I,III-3, (*b*) Cohn fraction VI and (*c*) Cohn fraction V. The columns were eluted with 0.15 M-NaCl/8 M-urea, the void volume is indicated by V_0 and the totally included volume by V_1.

Ethanol was removed by rotary evaporation from each of the three Cohn fractions. The remaining aqueous solutions were dialysed against water (2 × 2 litres for 24 h at 4°C), freeze-dried and dissolved in approx. 5 ml of 0.15 M-NaCl/5.4 mM-trisodium citrate. Aliquots (500 μl) of each fraction were subjected to chromatography on Sephacryl S-200 before and after digestion with neuraminidase (MacNicoll *et al.*, 1978). Analysis of the fractions for radioactivity (Fig. 2) showed that digestion with neuraminidase resulted in the appearance of a peak of radioactivity that coincided with the included volume of the column and accounted for 28.8 and 24.0% of the total radiolabel in Cohn fractions VI and V respectively. This low-molecular-weight material was not present in the undigested samples. Digestion with neuraminidase of Cohn fraction I,III-3 did not release any radiolabelled low-molecular-weight material and hexosamine analysis of a sample (2 ml) of this fraction showed that 97.5% of the radioactivity was present as [^{3}H]galactosamine.

Cellulose ion-exchange chromatography of radioactive components in plasma

Ion-exchange chromatography on DE-52 DEAE-cellulose is used to separate chondroitin 4-sulphate from plasma glycoproteins. When applied to the column in 0.15 M-NaCl, the plasma glycoproteins are not retained, and chondroitin 4-sulphate itself is eluted by 0.4–0.6 M-NaCl. Low-molecular-weight sugars are also washed through the column in 0.15 M-NaCl and are separated from the glycoproteins by subsequent gel filtration. A typical elution profile from DE-52 DEAE-cellulose of a plasma sample (2 ml) obtained after 5 h perfusion with preparation II is shown in Fig. 3. The proportions of radioactivity not retained by the column or eluted between 0.4 and 0.6 M-NaCl for this and other plasma samples are shown in Table 4.

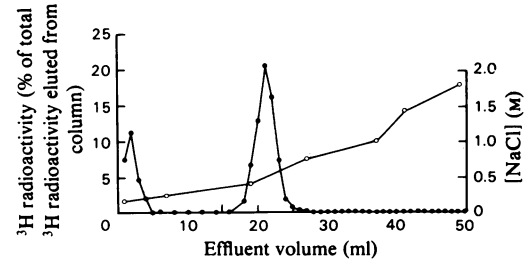

Fig. 3. *Chromatography on DE-52 DEAE-cellulose of plasma obtained after 5 h liver perfusion with [^{3}H]-chondroitin 4-sulphate*

The elution profile shows the distribution of radioactivity (●) in plasma after perfusion with chondroitin sulphate (preparation II). The column (110 mm × 9 mm) was eluted with an NaCl gradient (○) in 8 M-urea.

Table 4. *Ion-exchange chromatography on DE-52 DEAE-cellulose for separation of [^3H]chondroitin 4-sulphate and [^3H]labelled plasma glycoproteins*

For details see the text.

	Sample applied to column	Radioactivity not retained by column in 0.15 M-NaCl	Radioactivity eluted by 0.4–0.6 M-NaCl
Perfusion with preparation:			
II	Plasma (5 h)	24.5	75.5
III	Plasma (8 h) (after dialysis)	24.4	75.6
IV	Plasma (8 h)	29.5	70.5

After each perfusion, the radioactively labelled materials that eluted from a DE-52 DEAE-cellulose column between 0.4 and 0.6 M-NaCl were subjected to hexosamine analysis. In every case the radioactivity was associated with galactosamine.

The fractions containing radioactivity that were not retained by a DE-52 DEAE-cellulose column (derived from preparation II) were pooled and a sample (500 μl) was subjected to chromatography on Sephadex G-200. The majority of the radioactivity (60.6%) was associated with polymeric material and 26.2% of radiolabel in the pooled fraction was eluted by the total included volume of the column. Presumably the latter corresponds to the labelled material of low molecular weight detected by gel chromatography of unfractionated plasma.

After ion-exchange chromatography of plasma obtained after perfusion with preparation IV, the fractions containing radioactivity that was not retained on the column in 0.15 M-NaCl were pooled, dialysed against water and freeze-dried. Acid hydrolysis of this non-diffusible radioactivity and hexosamine analysis showed the radioactivity to be associated with glucosamine. However, the amount of radioactivity recorded was less than three times that of the background value and the possibility of some radiolabelled galactosamine could not be excluded.

Analysis of plasma after the incubation of [^3H]chondroitin 4-sulphate preparations with homologous heparinized blood

After incubation of control blood with each preparation of [^{3}H]chondroitin 4-sulphate, the analysis of plasma by ion-exchange chromatography on DE-52 DEAE-cellulose showed that all the radioactivity was eluted by a salt concentration of between 0.4 and 0.6 M-NaCl. Ethanol fractionation showed that 94–97% of the radioactivity in plasma was associated with Cohn fractions I,III-3; only trace amounts of radiolabel were distributed throughout the other fractions.

Digestion with neuraminidase of plasma, after control incubations of homologous rat blood with [^{3}H]chondroitin 4-sulphate preparations, did not release any low-molecular-weight material.

The rate of metabolism of [^3H]chondroitin 4-sulphate by the isolated perfused rat liver

The results demonstrate that after absorption and degradation of [^{3}H]chondroitin 4-sulphate by the liver, radiolabelled metabolites are secreted into the plasma either as polymeric glycoproteins or as low-molecular-weight compounds containing hexosamines. The rate of hepatic degradation of chondroitin sulphate preparations and the efficiency with which the ^{3}H-labelled monomers are utilized for the biosynthesis of ^{3}H-labelled glycoproteins can be compared for each perfusion by calculating the total amount of ^{3}H-labelled metabolites over the whole perfusion period (Table 5).

Discussion

The work described in the present study was undertaken in an attempt to establish the metabolic fate of *N*-acetylgalactosamine residues arising from the hepatic degradation of chondroitin 4-sulphate chains. During the course of perfusion of rat liver with chondroitin 4-sulphate labelled specifically in the hexosamine residues, three labelled components were identified in the plasma, chondroitin 4-sulphate itself, a low-molecular-weight component and glycoproteins. The newly synthesized polymers secreted into the plasma were distinguished from chondroitin 4-sulphate by ion-exchange chromatography and fractionation according to the Cohn scheme. The glycoprotein nature was confirmed by hydrolysis with neuraminidase and susceptibility to proteolytic digestion with papain (A. D. MacNicoll & C. G. Curtis, unpublished work). Cohn fractions V and VI contained the majority of the glycoproteins labelled with residues that originated from chondroitin 4-sulphate. These fractions include the α_1 acid glycoproteins, which have high proportions of hexosamine and sialic acid and therefore might be expected to be most labelled. In this respect the distribution of label is similar to that reported with free *N*-acetylgalactosamine as a precursor (MacNicoll *et al.*, 1978). This suggests that the *N*-acetylgalactosamine residues in the chondrotin 4-sulphate

Table 5. *Recovery of ^{3}H in plasma as polymeric (excluding chondroitin 4-sulphate) or low-molecular-weight material after perfusion of isolated rat livers with [^{3}H]chondroitin 4-sulphate*

Abbreviation: N.D., not determined.

Perfusion with preparation:	Administered dose of chondroitin 4-sulphate (μmol of hexosamine)	Duration of perfusion (h)	Liver weight (g)	^{3}H radioactivity recovered in plasma (% of administered dose)	Radiolabelled hexosamine as low-molecular-weight material (nmol)	Radiolabelled hexosamine as polymer (nmol)	Total radiolabelled hexosamine secreted by the liver (nmol)	Total rate of secretion of radiolabelled hexosamine (nmol/h per g of liver)	Rate of incorporation of radiolabelled hexosamine into polymer (nmol/h per g of liver)
I	4.7	5	9.9	73.0	764	N.D.	764	15.5	N.D.
II	4.1	5	11.7	83.2	224	836	1060	18.1	14.3
III	7.7	8	13.0	89.0	1450	1330	2780	26.7	12.9
IV	9.0	8	14.5	78.5	None	2080	2080	17.9	17.9

are activated and re-utilized directly for glycoprotein biosynthesis in this tissue.

White *et al.* (1965) by using [1-^{14}C]galactosamine to study plasma glycoprotein synthesis likewise have reported that most of the amino sugar was epimerized to the glucosamine derivative. Furthermore, hexosamine analysis of plasma glycoproteins synthesized by isolated rat hepatocytes, pulse-labelled with [1-^{14}C]glucosamine show a glucosamine/galactosamine ratio of 15:1 (T. Howe & P. J. Winterburn, unpublished work). Thus the apparent preferential labelling of the glucosamine probably reflects the fact that glucosamine predominates over galactosamine in rat plasma glycoproteins and that epimerization is a major event in the re-utilization of *N*-acetylgalactosamine.

The low-molecular-weight material released into the plasma in three of the four studies contained both radiolabelled galactosamine and glucosamine derivatives. The galactosamine in the perfusate was probably acetylated, although it was not investigated, because only a small proportion of *N*-acetylgalactosamine is deacetylated by the liver (MacNicoll *et al.*, 1978). This material derived from chondroitin 4-sulphate apparently diffuses out of the liver before being trapped by phosphorylation and used for polymer synthesis. An imbalance in the rate of chondroitin 4-sulphate catabolism versus the rate of reactivation of the sugars would lead to an accumulation of *N*-acetylgalactosamine. Another possibility is that the catabolism of chondroitin 4-sulphate and the synthesis of plasma glycoproteins occur in different cell types within the liver. This would necessitate the intercellular transport of monosaccharides and during the transfer some may pass into the plasma. Indeed, Kjellen *et al.* (1977) have reported that dermatan sulphate and chondroitin sulphate catabolism occur mainly in non-parenchymal cells, whereas plasma protein synthesis is carried out by hepatocytes (Crane & Miller, 1977; Struck *et al.* 1978).

The origin of labelled glucosamine in plasma is even less certain. The chondroitin 4-sulphate chains prepared by trypsin digestion are mainly dimers and tetramers attached to short peptides (Wood *et al.*, 1976), but a few short chains of keratan sulphate linked to asparagine residues in the peptide chain may also be present (F. S. Wusteman, unpublished work). Hexosamine analysis of the chondroitin sulphate showed that labelled glucosamine accounted for approx. 2% of the total radiolabelled hexosamines. Catabolism of these keratan sulphate chains may account for some of the radiolabelled glucosamine derivatives in plasma. Glucosamine may also arise from the degradation of labelled glycoproteins synthesized by the liver. The turnover time quoted for rat $_1$-glycoprotein fraction is 10–12h (Richmond, 1963). Therefore during 5–8h perfusion some

degradation by the liver would be anticipated. This would liberate glucosamine derivatives, which might diffuse into the plasma. Thus the variability in the appearance of low-molecular-weight material in plasma probably arises as a result of a number of factors: slight variations in the balance between catabolism and re-utilization, the nature of the starting material and, less likely, variation in the metabolism of the several isolated perfused liver preparations used.

The catabolic rate for chondroitin 4-sulphate was calculated from the rate of liberation of low-molecular-weight material into the plasma and the rate of utilization of the *N*-acetylgalactosamine for the synthesis of hexosamine and sialic acid residues in secreted glycoproteins. The values for the four studies ranged from 15.5 to 26.7nmol of hexosamine equivalents/h per g of liver. By contrast, the rate of consumption of the liberated *N*-acetylgalactosamine residues for polymer synthesis ranged between 12.9 and 17.9nmol of hexosamine equivalents/h per g of liver. These values fall short of the total demand for hexosamine and sialic acid by the liver. Richmond (1963) investigated the synthesis of an α_1-glycoprotein fraction by the isolated perfused rat liver and calculated the synthetic rate to be 5mg/kg of liver per min. Since the hexosamine plus sialic acid content was 25% this requires an amino sugar consumption of approx. 75nmol/g per h for this particular fraction alone. Thus the hexosamine provided by the catabolism of chondroitin sulphate probably supplemented but did not replace the synthesis *de novo* of hexosamines.

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