Use of radioactive glucosamine in the perfused rat liver to prepare α_1 -acid glycoprotein (orosomucoid) with ^3H - or ^{14}C -labelled sialic acid and *N*-acetylglucosamine residues

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1. A method was developed whereby [$1\text{-}^{14}\text{C}$]glucosamine was used in a perfused rat liver system to prepare over 2 mg of α_1 -acid glycoprotein with highly radioactive sialic acid and glucosamine residues. 2. The liver secreted radioactive α_1 -acid glycoprotein over a 4–6 h period, and this glycoprotein was purified from the perfusate by chromatography on DEAE-cellulose at pH 3.6. 3. The sialic acid on the isolated glycoprotein had a specific radioactivity of 3.1 Ci/mol, whereas the glucosamine-specific radioactivity was 4.3 Ci/mole. The latter amino-sugar residues on the isolated protein were only 13-fold less radioactive than the initially added [$1\text{-}^{14}\text{C}$]glucosamine. Orosomucoid with a specific radioactivity of 31.3 $\mu\text{Ci}/\text{mg}$ of protein was obtainable by using [$6\text{-}^3\text{H}$]glucosamine. 4. The amino acid composition of the purified orosomucoid was comparable with that found by others for the same glycoprotein isolated from rat serum. A partial characterization of the carbohydrate structure was done by sequential digestion with neuraminidase, β -D-galactosidase and β -D-hexosaminidase. 5. Many other radioactive glycoproteins were found to be secreted into the perfusate by the liver. Thus this experimental system should prove useful for obtaining other serum glycoproteins with highly radioactive sugar moieties.

The most common method for studying the metabolism of a biological compound is to label the molecule with radioisotope. My laboratory has been investigating how lysosomes function in cells, especially how hepatic lysosomes degrade serum glycoproteins. We have focused our experiments on the liver's system for receptor-mediated uptake of asialoglycoproteins that was discovered by Ashwell & Morell (1974). Our studies were done with desialylated commercial fetuin that had been radioactively labelled in its peptide component. Both ^{125}I -labelled-tyrosine- and [^{14}C]methyl-lysine-containing asialofetuin were very useful for characterizing lysosomal proteolysis of these complex macromolecules in the perfused rat liver and *in vivo* (LaBadie *et al.*, 1975, 1976; Dunn *et al.*, 1979, 1980). Many other workers also have used ^{125}I -labelled glycoproteins in similar studies (Tolleshaug *et al.*, 1977; Charlwood *et al.*, 1979; Hubbard *et al.*, 1979).

In contrast with the relative ease of labelling the peptide there are only a few methods by which to incorporate radioactivity into the carbohydrate residues of isolated glycoproteins, and, as a result,

much less information is known about the physiological breakdown of their sugar chains. [$6\text{-}^3\text{H}$]Galactose residues can be made on asialoglycoproteins by use of galactose oxidase and NaB^3H_4 (Morell *et al.*, 1966; Gregoriadis *et al.*, 1970). ^3H can also be incorporated into sialic acid residues of serum glycoproteins by selective periodate oxidation of this 9-carbon ketose at C-7, -8 and -9, followed by reduction of the aldehydes formed at these positions with NaB^3H_4 (Van Lenten & Ashwell, 1971). Depending on the amount of periodate used, either a 7- or 8-carbon analogue of sialic acid can be prepared on the carbohydrate chains. Since the modified residues are somewhat resistant to enzymic hydrolysis (Suttajit & Winzler, 1971), the metabolism of these labelled glycoproteins would not be expected to be entirely physiological. Hickman *et al.* (1970) and Van Den Hammer *et al.* (1970) enzymically desialylated caeruloplasmin and then re-incorporated various amounts of radioactive sialic acid into the asialo-caeruloplasmin using CMP ^{14}C -labelled sialic acid and a sialyltransferase prepared from liver microsomes. More recently, procedures have been de-

scribed for incorporating radioactive raffinose (Van Zile *et al.*, 1979) and sucrose (Pittman *et al.*, 1979; DeJong *et al.*, 1981) into the peptide component of proteins, glycoproteins and lipoproteins, but these oligosaccharides are not substrates for lysosomal glycosidases and the labelling technique is more suitable for studying overall degradation of the macromolecule (Baynes & Thorpe, 1981).

Many diseases are known in which there is defective lysosomal catabolism of polymeric carbohydrates. The organelles become compacted with this class of compound owing to the genetic lack of a lysosomal glycosidase. However, there still are not much biochemical data that describe the process by which lysosomes normally degrade macromolecular carbohydrates. We therefore have developed a procedure for preparing orosomucoid labelled with radioactive sialic acid and *N*-acetylglucosamine residues to be used for such metabolic studies. The method involves adding [$6\text{-}^3\text{H}$]- or [$1\text{-}^{14}\text{C}$]-glucosamine to an isolated perfused rat liver followed by purification of the radioactive glycoprotein that is newly synthesized and secreted into the perfusate.

Experimental

Liver perfusion

As previously reported (LaBadie *et al.*, 1975, 1976; Dunn *et al.*, 1979, 1980) rat livers were perfused by using a modification of the system originally developed by González de Galdeano *et al.* (1973). Male Wistar rats weighing between 250 and 370 g were starved for 24 h and anaesthetized by an intraperitoneal injection of sodium pentobarbital. The abdominal cavity was cut open and the bile duct was cannulated with PE-10 intramedic tubing (Clay-Adams). An 18-gauge cannula was inserted surgically into the hepatic portal vein and tied in place. The liver was quickly excised from the animal and perfused briefly at 10 ml/min to remove all erythrocytes from the tissue. The organ was then hung from the mounted cannula over a collecting funnel so that the effluent perfusate returned to heat and O_2 exchangers, and the flow of perfusion medium was increased to 45 ml/min. The cyclic system consisted of a Masterflex pump (Cole-Parmer Instrument Co.), a heat exchanger made from a microglass condenser maintained at 35°C by a Haake circulator bath and an O_2 exchanger. The latter component, which also served as a perfusate reservoir, contained gas-permeable hollow fibres mounted as a semi-circular loop through a 100 ml plastic beaker assembly (Bio-Rad Laboratories). Pure O_2 was passed through the fibres to keep the perfusate saturated with O_2 . The perfusate medium was the same as described previously (Dunn *et al.*, 1979) except 2% (w/v) bovine serum albumin

(fraction V; Miles Laboratory) replaced PVP-40 and the solution contained 10 mM-pyruvate and 0.8 mM-sodium palmitate instead of 5 mM-glucose. The fatty acid was added by mixing 1 vol of 8 mM-sodium palmitate in 0.9% NaCl with 9 vol. of perfusate. Of the 100 ml perfusate volume, 8 ml were lost/h because of sampling and evaporation during the 4–5 h period of perfusion. This amount of fluid was replaced by infusing new perfusate solution that contained an additional 12.6 mM-pyruvate at the same rate with a Sage syringe pump.

Labelling with radioactive glucosamine

Both [$6\text{-}^3\text{H}$]glucosamine (New England Nuclear Corp.) and [$1\text{-}^{14}\text{C}$]glucosamine (Rosechem Products, Los Angeles, CA, U.S.A.) were found to work well for these experiments. The procedure described is for [$1\text{-}^{14}\text{C}$]glucosamine (sp. radioactivity 56 Ci/mol). Turpentine (1 ml) was injected intramuscularly into the rat 40 h before isolating the liver, since this treatment has been shown to enhance liver synthesis of serum glycoproteins (Jamieson *et al.*, 1975; Lombart *et al.*, 1980). After the liver had been equilibrated for 30 min in the perfusion system, 125 μCi of [$1\text{-}^{14}\text{C}$]glucosamine in 0.25 ml of 50% ethanol was added. At appropriate times 0.1 ml portions of the perfusate were removed to be analysed for their content of acid-soluble and acid-precipitable radioactivity. At 60 min another 125 μCi of [$1\text{-}^{14}\text{C}$]glucosamine was added. The perfusion with radioactive sugar was continued for a total time of 4–6 h.

Isolation of radioactive orosomucoid

Purification of the labelled α_1 -acid glycoprotein from the perfusate was based on the procedure of Shibata *et al.* (1977). The approx. 100 ml of perfusate was thoroughly dialysed three times against a solution that contained 0.11 M-acetic acid and 0.01 M-sodium acetate (final pH 3.6). The dialysed perfusate was then pumped on to a column (1 cm $\times$ 12 cm) of DEAE-cellulose pre-equilibrated and washed with the same pH 3.6 buffer at 20 ml/h. After a total of 120 ml had been collected, 80 ml of starting acetate buffer that contained 15 mM-NaCl in addition (low-salt buffer) was run through the column. The column was finally eluted with 40 ml of starting buffer that contained 0.15 M-NaCl (high-salt buffer). The radioactive orosomucoid removed from the column by this high-salt buffer was dialysed thoroughly against distilled water, freeze-dried and retained for further analysis. Of the total radioactive material in the dialysed perfusate (approx. 1×10^8 c.p.m.), 80% was eluted in the run-through volume, 5% in the low-salt peak and 15% (orosomucoid) was eluted in the high-salt peak.

Assays

Protein was assayed by the method of Miller (1959). Amino acid analyses were done on a Dionex amino acid analyser using fluorimetric detection of amino groups. Sialic acid was determined by the thiobarbiturate procedure of Warren (1959). Radioactivity was counted as previously described (LaBadie *et al.*, 1976).

Results

Incorporation of [1-¹⁴C]glucosamine into secreted glycoproteins

Glucosamine is an excellent precursor for serum glycoproteins made by the liver (Macbeth *et al.*, 1965). Fig. 1 shows the uptake of [1-¹⁴C]glucosamine by the perfused organ and its secretion of labelled glycoprotein into the perfusate. The liver removed in 4 h approx. 67% of the 4.5 μ mol (250 μ Ci) of glucosamine initially added to the perfusate and released radioactive glycoprotein into the perfusion

medium at a constant rate beginning 1 h after addition of the labelled sugar. A similar short lag-period for glycoprotein secretion has been reported by others (Van Kooij & Poort, 1980). The glycoproteins in the final perfusate contained 24% of the [1-¹⁴C]glucosamine dose. However, the ability of the liver to incorporate glucosamine into medium glycoprotein was not saturated when using this initial quantity of radioactive hexosamine (Fig. 2). Almost linear incorporation of the sugar was observed on adding up to 10 μ mol of [1-¹⁴C]glucosamine.

Purification of α_1 -acid glycoprotein from the perfusate

The final radioactive perfusate was thoroughly dialysed and chromatographed on a small column of Sephacryl 200 (Fig. 3). The perfusion medium initially contained 2% bovine serum albumin and there was no distinct separation of radioactive substances from this major protein by gel-permeation chromatography. However, a number of radioactive glycoproteins were found to be produced and secreted by the perfused liver on further analysing

Fig. 1. Incorporation of [1-¹⁴C]glucosamine into secreted glycoproteins by the perfused rat liver

A liver was perfused with 125 μ Ci of [1-¹⁴C]-glucosamine (sp. radioactivity 56 Ci/mol) according to the procedure described in the Experimental section. At 1 h a second addition of radioactive glucosamine was made. At the indicated times 5 μ l of the perfusate were removed, diluted with 195 μ l of fresh perfusate medium and precipitated with 1.0 ml of 4% phosphotungstic acid in 2 M-HCl at room temperature. The precipitate was centrifuged and washed with 0.5 ml of acid solution. The final pellet was dissolved in 1.0 ml of 1 M-NaOH and used to determine total-perfusate acid-precipitable radioactivity (○) as previously described (Dunn *et al.*, 1979). A 0.25 ml sample of the combined supernatant solutions was also analysed for its content of acid-soluble radioactivity (●).

Fig. 2. Effect of glucosamine concentration on the incorporation of [1-¹⁴C]glucosamine into perfusate glycoproteins

Livers from rats were perfused for 5 h by the procedure described in the Experimental section. Various amounts of unlabelled glucosamine were mixed with [1-¹⁴C]glucosamine (sp. radioactivity 56 Ci/mol) to obtain the final level of the total glucosamine added to the perfusate. At the end of the experiment the total amount of acid-precipitable radioactivity in the dialysed perfusate was measured as described in the legend for Fig. 1. The amount of glucosamine incorporated into glycoprotein (●) was calculated on the basis of the specific radioactivity of the initially added sample of [1-¹⁴C]glucosamine.

Fig. 3. Analysis of radioactive glycoproteins in a liver perfusate using Sephacryl 200 chromatography

A 0.9 ml sample of dialysed perfusion medium (9.7×10^5 c.p.m./ml) was obtained from a liver perfused for 6 h and applied to a column (1 cm $\times$ 44 cm) of Sephacryl 200. The column was equilibrated and eluted with 0.05 M-sodium phosphate buffer, pH 7.4, that contained 0.1 M-NaCl. Fractions (0.75 ml) were collected every 5 min and analysed for total radioactivity (●) and A_{280} (○). Standard elution volumes from the column were determined with Blue Dextran (V_0), cytochrome *c* and glucose (V_g). Fractions shown with an asterisk were further analysed by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis (see Fig. 4).

various column fractions by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis and fluorography (Fig. 4).

Most procedures for purifying orosomucoid depend on the very low pI of the glycoprotein. [$1\text{-}^{14}\text{C}$]Glucosamine-labelled orosomucoid therefore was purified from the dialysed perfusate by chromatography on DEAE-cellulose at pH 3.6 (Fig. 4). Significant amounts of only two proteins adsorbed to DEAE-cellulose at this low pH, and they were separated from one another by eluting the column with pH 3.6 buffer of various NaCl concentrations. A single band was observed in the high-salt fraction (orosomucoid) when it was analysed by fluorography (Fig. 4) and protein staining (not shown) after being electrophoresed in sodium dodecyl sulphate/polyacrylamide gels. In three separate experiments 1.3, 1.9 and 2.3 mg of orosomucoid protein (assay method of Miller, 1969) were isolated. The average specific radioactivity of the three samples was $4.0 \mu\text{Ci}/\text{mg}$ of protein. By using 1 mCi of [$6\text{-}^3\text{H}$]glucosamine (19 Ci/mmol) similar quantities of orosomucoid were obtained in the high-salt fraction, but having a much higher specific radioactivity ($31.3 \mu\text{Ci}/\text{mg}$ of protein). The contaminat-

Fig. 4. Purification of [$1\text{-}^{14}\text{C}$]glucosamine-labelled orosomucoid as analysed by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis and fluorography

A perfused rat liver was treated with $250 \mu\text{Ci}$ of [$1\text{-}^{14}\text{C}$]glucosamine for 6 h as described in the Experimental section. A sample of the dialysed perfusate was chromatographed on Sephacryl 200 (see the legend to Fig. 3) and the remainder was run on a DEAE-cellulose column according to the details given in the Experimental section. Purified [^{14}C]orosomucoid was eluted from the latter column in the high-salt fraction (HS) after a contaminant had been removed with low-salt buffer (LS). Both the high- and low-salt fractions from the DEAE-cellulose column and fractions 26 and 30–33 from the Sephacryl 200 column were analysed by a modification of the sodium dodecyl sulphate/polyacrylamide-gel-electrophoresis system previously described (Dunn *et al.*, 1979). A thinner gel (1.5 mm thick) was used and glycoproteins were fixed for 1 h in 2 M-HCl containing 4% phosphotungstic acid followed by overnight soaking in a solution made of 25% (v/v) propan-2-ol and 10% (v/v) acetic acid. The washed gel was prepared for fluorography using Enhance (New England Nuclear Corp.) according to the manufacturer's instructions. The gel was dried on Whatman 3MM paper, placed over X-Omat AR film (Eastman Kodak Co.) and exposed at -70°C for 2 weeks before development. Each fraction contained approx. 5000 c.p.m. The molecular-weight standards were [^{14}C]methylated proteins obtained from Amersham Corp.

ing fraction that was eluted from DEAE-cellulose by low salt (Fig. 4) contained more protein (average of 4.6 mg) with less ^{14}C radioactivity ($0.6 \mu\text{Ci}/\text{mg}$ of protein) and was not purified completely.

Characterization of [$1\text{-}^{14}\text{C}$]glucosamine-labelled orosomucoid

The amino acid composition of the purified ^{14}C -labelled orosomucoid was measured and com-

pared with that found by other workers, who had isolated the glycoprotein from rat serum (Table 1). There was generally close agreement for the four analyses. Our results were most similar to the amino acid composition reported by Charlwood *et al.* (1976), who isolated orosomucoid by a completely different procedure. The largest difference between the compositions was in the number of residues of methionine and tyrosine, the amino acids of lowest concentration in the two proteins. A recent report by this same laboratory (Charlwood *et al.*, 1979) showed their purified glycoprotein to be digested poorly *in vitro* by extracts of lysosomal proteinases, an observation we have also made using our protein labelled with either [1-¹⁴C]glucosamine or ¹²⁵I.

We also determined how much sialic acid and glucosamine was present in the purified [1-¹⁴C]-glucosamine-labelled glycoprotein (Table 1). Our glycoprotein as well as two of the other three preparations of orosomucoid contained 14–15 residues of sialic acid per molecule. However, those two rat serum orosomucoids contained 23–25 residues of glucosamine per molecule, whereas our sample isolated from the perfused liver had a somewhat higher content of this amino sugar (30 residues per molecule). Thus the respective sialic acid/glucosamine ratios would be either 3–5 or 1–2 for the purified glycoproteins. The 1–2 ratio of our pre-

paration fits the biantennary-type of carbohydrate structure, which has been reported to be the major structure on total glycoproteins of rat serum [71% of the carbohydrate recovered from rat serum total glycopeptides (Finne & Krusius, 1979)].

Galactosamine was quantified along with glucosamine in the amino acid-analysis system used for the present study, and none of this amino sugar was found present in the hydrolysate of the labelled glycoprotein. This absence implies that rat orosomucoid, like the human protein (Fournet *et al.*, 1978), does not contain *O*-glycosidically linked carbohydrate chains. By using a stream splitter on the ion-exchange column, samples of each amino acid fraction were analysed for any radioactivity, which was found exclusively with the peak of glucosamine (results not shown). The specific radioactivity of the sialic acid in the ¹⁴C-labelled orosomucoid was 3.1 Ci/mol, whereas that of glucosamine was 4.3 Ci/mol. This labelling difference can be explained by the known metabolic precursor-product relationship between glucosamine and *N*-acetylneuraminic acid.

A partial characterization of the structure of the sugar component of the [¹⁴C]orosomucoid was done by treating the purified glycoprotein sequentially with neuraminidase, β -D-galactosidase and β -D-hexosaminidase (Fig. 5). Per molecule of glyco-

Table 1. *Amino acid and amino sugar composition of rat α_1 -acid glycoproteins*

The residues of amino acids and sugars were calculated on the basis of glycoproteins having a molecular weight of 43 000.

Source of α_1 -acid glycoprotein	Results of	Amino acid or sugar content (residues/mol)			
		Rat serum			Perfused liver The present paper
Amino acid or sugar	...	Friesen & Jamieson (1980)	Nagashima <i>et al.</i> (1980)	Charlwood <i>et al.</i> (1976)	
Asp		22.9	25	34.9	34.4
Thr		17.2	21	26.2	28.2
Ser		13.7	9	11.6	16.4
Glu		28.7	35	40.9	50.1
Pro		9.8	10 (+Cys)	13.5	—
Gly		12.1	11	17.0	15.5
Ala		13.7	12	17.5	20.7
Val		13.1	14	13.9	10.5
Met		3.1	3	4.6	1.2
Ile		10.4	11	13.7	9.9
Leu		17.1	22	25.2	24.1
Tyr		5.8	6	4.9	2.9
Phe		11.4	15	17.4	14.3
Lys		17.1	22	29.7	21.2
His		4.3	4	5.3	5.7
Arg		6.2	6	8.7	7.6
Trp		4.0	4	4.0	—
GlcN		23.0	24.5	15	30.2
GalN		—	—	—	0
NeuAc		15.4	14.3	7	14.1

Fig. 5. Digestion of $[1-^{14}\text{C}]$ glucosamine-labelled orosomucoid with glycosidases

A 1.5 mg sample of purified $[1-^{14}\text{C}]$ glucosamine-labelled orosomucoid (2.3×10^6 c.p.m.) was digested with 100 μl of insolubilized neuraminidase (Sigma Chemical Co.; type VI-A) in 0.5 ml of 0.1 M-sodium acetate buffer, pH 4.5, for 3 days. The released radioactive sialic acid was separated from the asialoglycoprotein by passage through the same column of Sephacryl 200 as described in the legend to Fig. 3, except that the eluent was water. The glycoprotein was freeze-dried, redissolved in 0.125 ml of 0.1 M-sodium citrate buffer, pH 4.7, and digested for 2 days with 0.8 unit of jack-bean β -D-galactosidase (Li & Li, 1972). The reaction was again run on the Sephacryl 200 column and the fractions containing radioactive glycoprotein were freeze-dried. This material was finally digested with 1.2 units of jack-bean β -D-hexosaminidase (Sigma Chemical Co.) for 2 days in 0.125 ml of the pH 4.7 citrate buffer. All three reactions were done at 37°C in the presence of a drop of toluene. Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis and fluorography were performed as described in the legend to Fig. 4 using approx. 5000 c.p.m. per sample. Abbreviations used: A, orosomucoid; B, neuraminidase-treated orosomucoid; C, β -D-galactosidase-treated sample B; D, β -hexosaminidase-treated sample C.

protein 14.6 molecules of sialic acid and 10.1 of galactose were released by the first two glycosidases and the mobility of the degraded substrate increased accordingly during sodium dodecyl sulphate/polyacrylamide-gel electrophoresis. However, very little *N*-acetylglucosamine was then removed by jack-bean β -D-hexosaminidase (20% of the total radioactivity). The structure of this resistant carbohydrate material requires further investigation.

Discussion

The liver, which is the source of most serum glycoproteins (Spiro, 1959; Sarcione, 1962; Robin-

son *et al.*, 1964), is very efficient at labelling the carbohydrate component of these macromolecules with radioactive glucosamine. Macbeth *et al.* (1965) showed that the rat could incorporate in 5 h almost 25% of intraperitoneally injected $[1-^{14}\text{C}]$ glucosamine into residues of both hexosamine (16.2%) and *N*-acetylneuraminic acid (7.9%) in the total fraction of serum glycoproteins. We have been able to accomplish this same level of incorporation with the perfused rat liver. The isolated organ offers the advantage that the newly secreted radioactive glycoproteins are not diluted as they are *in vivo* by the high concentrations of the same proteins already in the serum. Thus Macbeth *et al.* (1965) reported the specific radioactivity of hexosamine residues made on serum glycoproteins in the rat to be 170-fold less than that of the starting sugar, whereas the specific radioactivity of $[1-^{14}\text{C}]$ glucosamine added to the perfusate in our study was lowered just 13-fold in the purified α_1 -acid glycoprotein. The starting perfusion medium did contain a high level of bovine serum albumin. However, it is unlikely that bovine α_1 -acid glycoprotein was a significant contaminant in this commercial product, since α_1 -acid glycoprotein is normally isolated from fraction V supernatant (Hao & Wickerhauser, 1973) and not fraction V precipitate, the source of serum albumin. If any significant amount of unlabelled bovine α_1 -acid glycoprotein had co-purified with the rat glycoprotein eluted from DEAE-cellulose (Fig. 4), one also would expect to have found a much greater dilution of the $[1-^{14}\text{C}]$ glucosamine isotope in the final product.

As expected, the added radioactive glucosamine did not appear to cause indirect labelling of neutral sugars on the glycoprotein. Thus galactose enzymically released from the purified α_1 -acid glycoprotein (Fig. 5) was isolated and found to be free of radioactivity. It is also improbable that any mannose or fucose residues were labelled in the present study. Such incorporation of radioisotope would have required metabolic conversion of the added $[1-^{14}\text{C}]$ glucosamine into mannose 6-phosphate via the intermediate fructose 6-phosphate, which the perfused liver more likely would convert to glucose. Indeed, in a preliminary experiment in which $[2-^3\text{H}]$ mannose itself was added to the perfusate, little radioactivity was incorporated into purified orosomucoid, even though this hexose was taken up by the liver.

Recently there has been a growing interest in the non-enzymic incorporation of labelled sugars into serum glycoproteins. However, it is improbable that this non-biological process occurred with $[1-^{14}\text{C}]$ -glucosamine in the perfused liver. Thus, these chemical reactions are reported to be slow, generally taking days (Day *et al.*, 1979), whereas the perfusion experiments lasted only 4–5 h. The radio-

active sugar used for such non-enzymic incorporation (generally glucose) is also added at a 100-fold higher concentration (5 mM) than the [$1\text{-}^{14}\text{C}$]glucosamine present in the perfusion medium (approx. 0.045 mM).

Other radioactive sugars may be useful for preparing serum glycoproteins that contain a high level of radioisotope. MacNicol *et al.* (1978) used *N*-acetyl-D- $[1\text{-}^{14}\text{C}]$ galactosamine for the biosynthesis of glycoproteins in the perfused rat liver. They found that this sugar was also incorporated mainly as radioactive sialic acid and *N*-acetylglucosamine. *N*-Acetyl-D- $[1\text{-}^{14}\text{C}]$ glucosamine itself does not appear to be well suited as a sugar precursor for serum glycoproteins, probably because of its poor entry into cells (McGarrahan & Maley, 1962; Richmond, 1963). The amino sugar D- $[1\text{-}^{14}\text{C}]$ mannosamine is commercially available and potentially could be metabolized to radioactive *N*-acetylneuraminic acid. However, Raisys & Winzler (1970) found this sugar was not incorporated into serum glycoproteins when injected into rats, even though it was temporarily taken up by the liver. Friesen & Jamieson (1980) more recently have injected rats with D- $[1\text{-}^{14}\text{C}]$ -mannose to investigate the biosynthesis of orosomucoid. Radioactive galactose (Richmond, 1963) and fucose (Bekesi & Winzler, 1967; Kaufman & Ginsburg, 1968) have also been useful for studying synthesis of glycoproteins in cells and tissues. Although we are not certain of the fucose content of our sample of rat α_1 -acid glycoprotein, its presence has been reported by Shibata *et al.* (1977). Residues of this 6-deoxy sugar may be the cause of our failure to release much *N*-acetylglucosamine from the agalacto-derivative with jack-bean β -D-hexosaminidase (Fig. 5; Yamashita *et al.*, 1979). It is likely that other sugar residues on α_1 -acid glycoprotein could be labelled effectively by using the appropriate radioactive monosaccharide in our perfused liver system.

These experiments show that the perfused liver can be used to obtain a large quantity of α_1 -acid glycoprotein with high specific radioactivity in its sialic acid and hexosamine moieties. Turpentine-induced inflammation appeared to be important for our obtaining consistent yields of this glycoprotein. John & Miller (1969) were able to induce a perfused rat liver to synthesize even greater amounts of α_1 -acid glycoprotein. They used amino acids, somatotropin, insulin and cortisol in a perfusate that contained red blood cells to cause the formation of up to 21 mg of orosomucoid (measured immunologically) within 12 h. However, only 2–3 mg of the glycoprotein was produced in the initial 4 h of perfusion (the time period routinely used by ourselves), and an enhanced rate of synthesis occurred only during the last 8 h of their experiment.

The perfused liver makes and secretes many

glycoproteins besides α_1 -acid glycoprotein (Figs. 3 and 4) and this experimental system should be very useful for obtaining them labelled with radioactive sugars as well. A single radioactive component was found in fraction 26 of the Sephacryl 200 column eluate (Fig. 3). Its apparent molecular weight calculated from elution volume is approx. 150 000, yet its molecular size appears to be 35 000 when electrophoresed under denaturing and reducing conditions (Fig. 4). This result suggests that the native glycoprotein exists as a tetramer of the single highly radioactive component appearing on the sodium dodecyl sulphate/polyacrylamide gel in Fig. 4. We do not know the identity of this interesting glycoprotein. Considering the liver's impressive ability to make glycoproteins, it is surprising how little experimental work has been done recently on the processing of the carbohydrate component of these macromolecules using this tissue (Nagashima *et al.*, 1980; Friesen & Jamieson, 1980).

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