Biochemical Study of Cardiac Valvular Tissue

BIOSYNTHESIS *IN VITRO* OF HEXOSAMINE-CONTAINING SUBSTANCES IN BOVINE HEART VALVE

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1. Two distinct hexosamine-containing substances have been obtained from bovine cardiac valvular tissue which was incubated with labelled glucosamine. These were identified as mucopolysaccharides and glycopeptides respectively, both by the elution pattern from a Sephadex G-50 column and by chemical analysis. 2. In the mucopolysaccharide fraction about 80% of both the total hexosamine and total radioactivity were present; the galactosamine/glucosamine ratio of the amounts was about 1.36. 3. The glycopeptide fractions had about 20% of both total hexosamine and total radioactivity; the galactosamine/glucosamine ratio of amounts was about 0.44. In this fraction, over 15% of radioactivity was present in sialic acid. 4. In contrast with the concentrations of the several chemical components, there were remarkable differences in the biosynthetic activities among the four valves; the tricuspid valve had highest specific radioactivity in all components of both substances, followed then in turn by mitral, aortic and pulmonary valves. 5. Glucosamine in mucopolysaccharides had a rapid rate of turnover, followed then in turn by turnover rates of both glucosamine and galactosamine in glycopeptides, and of galactosamine in mucopolysaccharides. The possible significance of these findings is discussed.

The ground substance of connective tissue, including that of the heart valves, contains several glycoproteins, as well as mucopolysaccharides. Although much is known about the identification, extraction and characterization of the mucopolysaccharides of the valvular tissue of various species (Meyer & Rapport, 1951; Deiss & Leon, 1955; Meyer, Davidson, Linker & Hoffman, 1956; Moretti & Whitehouse, 1963; Torii, Bashey & Nakao, 1965; Toole & Lowther, 1965; Torii & Bashey, 1966; Lowther, Toole & Meyer, 1967; Toole & Lowther, 1968; Meyer, Preston & Lowther, 1969), little is known of their biosynthesis (Boström, Moretti & Whitehouse, 1963; Jacobson & Boström, 1964). In contrast, the glycoprotein of heart valve has been subject to some chemical study (Moczar & Moczar, 1967; Robert & Comte, 1968), but most glycoprotein components have not been well characterized as yet, nor has much been learned of their formation.

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Previous study in this laboratory has demonstrated that the heart valve has the capacity to produce its own glycoproteins locally, as well as mucopolysaccharides (Kanke, Bashey, Mori & Angrist, 1970). This same study showed that the carbohydrate moieties are more rapidly synthesized in glycoproteins than in mucopolysaccharides, and that the biosynthetic activities of both substances are higher in the aortic than in the pulmonary valve.

In the present paper, the results of more extended studies of both mucopolysaccharides and glycoprotein are described; also the findings in the four bovine heart valves are described and compared.

EXPERIMENTAL

Materials. Bovine hearts (8-10-year-old cows) were collected at the abattoir immediately after slaughter and transported to the laboratory in ice. Aortic, pulmonary, mitral and tricuspid valves were excised separately, avoiding muscle tissue; sections from all valves used were removed for routine histologic examinations. Minces from individual heart valves were prepared by using scissors to obtain fragments 2-3 mm in diameter. The minced

tissue was rinsed a few times with cold 0.9% NaCl to remove any contaminating blood.

Analytical methods. Hexosamine was determined by a modification (Gatt & Berman, 1966) of the Elson-Morgan reaction after hydrolysis of the sample in 2M-HCl at 100°C for 16h. Uronic acid was measured by the method of Bitter & Muir (1962) with glucuronolactone as standard; the value due to neutral sugars, which contributed less than 5% of the total colour yield, was subtracted. Sialic acid analysis was performed by the thiobarbituric acid method according to Warren (1959) after hydrolysis in 0.05M-H₂SO₄ at 80°C for 1h. It is

possible that these conditions give an underestimate of the sialic acid content of the material (see Gibbons, 1963). Neutral sugars were determined by the phenol-H₂SO₄ reaction (Hodge & Hofreiter, 1962). Fucose was measured by the cysteine-H₂SO₄ method (Dische & Shettles, 1948). Radioactivity was measured in a Packard Tri-Carb liquid-scintillation spectrometer, with Bray's (1960) solution.

Incubation of minced tissue with labelled glucosamine. Approx. 1g portions of the wet minced individual heart valve were placed in 25ml Erlenmeyer flasks containing 10ml of Krebs-Ringer bicarbonate buffer (DeLuca & Cohen, 1964), pH 7.4, with 100 μ Ci of D-[6-³H]glucosamine

Fig. 1. Profile obtained from papain-digested bovine heart valves from Sephadex G-50 column. Samples (80mg of dry fat-free tissue) were applied to the column (1.6cm $\times$ 70cm), previously equilibrated with 0.05M-ammonium acetate, and were eluted with the same buffer. Hexosamine was determined in 0.2ml of the eluate (total volume 2ml). Another 0.1ml was used for the determination of radioactivity in a liquid-scintillation counter. Contents of test-tubes corresponding to peaks I and II were combined and used for further analysis of constituents. ●, Hexosamine content; ○, radioactivity. Valve: (a) aortic; (b) pulmonary; (c) mitral; (d) tricuspid.

(specific radioactivity 1150 mCi/mmol; New England Nuclear Corp., Boston, Mass., U.S.A.), 500 μ g of streptomycin, and 500 units of penicillin (Krebs, 1950). The flasks were incubated with continuous shaking at 37°C in an atmosphere of O₂+CO₂ (95:5) for 6 h except in the case of the time-course study. At the end of the incubation period, the reaction mixture was chilled rapidly by placing it in ice-water and by adding cold 0.9% NaCl solution; the tissue was then washed with 0.9% NaCl several times.

Papain digestion. Before papain digestion, the valves were dehydrated with acetone, defatted with at least six changes of chloroform-methanol (1:1, v/v) under constant shaking at room temperature for two days, and then dried under reduced pressure over silica gel. Approx. 100 mg of the fat-free dried samples was mixed with 2 ml of 0.1 M-phosphate buffer, pH 6.4, containing 5 mM-cysteine hydrochloride, 5 mM-EDTA and 2 mg of papain (EC 3.4.4.10, twice crystallized, 30 mg of protein/ml of suspension; Sigma Chemical Co., St Louis, Mo., U.S.A.). The samples were then incubated at 60°C for 30 min after which a further 2 ml of the same papain solution was added, and the incubation was continued for another 24 h. The digests were dialysed in Visking tubing against running tap water for 24 h and then against distilled water with changes every 8 h for 2 days at 4°C for a total of 10000 vol. The dialysis residues were then centrifuged at 15000 rev./min for 30 min. The clear supernatant fluids from each valve were pooled to obtain sufficient material for chemical analysis. Portions of the combined samples were saved for hexosamine assay and for radioactivity determination, and the remainder was evaporated under reduced pressure at a temperature not exceeding 45°C.

Chromatography on a Sephadex G-50 column. The papain-digested dried aortic, pulmonary, mitral and tricuspid valve samples were dissolved respectively in a small amount (1–2 ml) of 0.05 M-ammonium acetate and each sample was then applied to a column (1.6 cm $\times$ 70 cm) of Sephadex G-50 (fine grade; Pharmacia Fine Chemicals Inc., Piscataway, N.J., U.S.A.) previously equilibrated with the same solution. The column was eluted with the same solution and fractions of 2 ml were collected every 18 min. Samples (0.2 ml) were withdrawn from each tube for assay of hexosamine. Additional portions (0.1 ml) were used for liquid-scintillation counting.

The two major peaks obtained from the column were each collected separately in dialysis bags as indicated by the vertical broken lines in Fig. 1 and dialysed against several changes of water. The contents of the dialysis bags were evaporated under reduced pressure and stored in the cold until used.

Paper chromatography of hexosamines and neutral sugars. Separation of glucosamine and galactosamine was achieved by paper chromatography (White, Shetlar, Shurley & Schilling, 1965). The HCl used to hydrolyse the sample was removed under reduced pressure over P₂O₅ and NaOH at 45°C. No corrections for possible losses under the conditions used for removing HCl have been applied. The dried hydrolysate was dissolved in a small amount of water and chromatographed on Whatman no. 1 paper by a descending technique with ethyl acetate-pyridine-water (12:5:4, by vol.) as solvent for 16 h at room temperature. After drying, the strips for the standards were stained with alkaline AgNO₃ (Trevelyan, Procter & Harrison, 1950), and corresponding spots of the

sample strips were removed and cut into small pieces. For measurement of radioactivities incorporated into both hexosamines, the pieces of paper were transferred to counting vials. The amounts of glucosamine and galactosamine on the paper were determined directly by a modification of the method of Neuhaus & Letzring (1957). Briefly, pentane-2,4-dione was added to the tube containing pieces of paper strips; the condensation products were extracted with 3-methylbutan-1-ol. This extract was then treated with Ehrlich's reagent.

Qualitative analysis of neutral sugars was also performed by use of the paper-chromatographic system described above.

Electrophoresis on cellulose acetate. Peak I, isolated by chromatography on Sephadex G-50, was subjected to electrophoresis on cellulose acetate strips (Gelman Instrument Co., Ann Arbor, Mich., U.S.A.) with acetic acid-pyridine buffer, pH 3.5, and voltage of 200 V/15 cm for 80 min at room temperature. The bands were visualized by dipping the strips into 0.1% Alcian Blue followed by a wash in 5% (v/v) acetic acid. Standard hyaluronic acid, chondroitin sulphate A, chondroitin sulphate C, dermatan sulphate and heparitin sulphate, all of which were a generous gift from Dr M. B. Mathews, were used for comparison purposes.

Column chromatography of sialic acid. To determine the radioactivity of sialic acid, the 0.05 M-H₂SO₄ hydrolysate was applied to a small column of Dowex 1 (X8; 200–400 mesh; formate form) as described by Spiro (1966). After the column was washed with water, the sialic acid was eluted with 0.3 M-formic acid. The formic acid was removed from this eluate by evaporation under reduced pressure at 45°C.

Amino acid analysis. Amino acid analysis was performed on the Beckman model 120 C amino acid analyser by the procedure of Spackman, Stein & Moore (1958) and Hubbard (1965) after hydrolysis with 6 M-HCl in sealed tubes at 110°C for 24 h under reduced pressure.

RESULTS AND DISCUSSION

Time-course of incorporation of labelled glucosamine. Minces of bovine heart valves *in vitro* were apparently able to incorporate labelled glucosamine. Incorporation was slow in the first 2 h of incubation, but then proceeded at a faster rate in the next 4 h (Fig. 2). The lag phase is characteristic of valvular tissue and may be due to its density and impermeability. Similar curves were found with individual valves in studies of collagen biosynthesis utilizing tritiated proline (Mori, Bashey & Angrist, 1967).

Fractionation of papain-digested material by Sephadex G-50. To separate roughly the papain-digested sample into mucopolysaccharides and glycopeptides, chromatography on Sephadex G-50 column was carried out, as suggested by Bates, Levene & Kodicek (1969).

Fig. 1 shows that two distinct radioactive hexosamine-containing peaks were obtained from the papain digests of all four valves. Peak I was a sharp peak eluted at the void volume of the Sephadex column, and peak II was a broad peak.

As described later, peaks I and II corresponded to mucopolysaccharides and glycopeptides respectively. In all four valves, 76–82% of the total hexosamine was present in peak I, the remainder was found in peak II (Table 1). These findings are different from the results reported by Lowther *et al.* (1967) who found that only 28–36% of the hexosamine was present in the acid mucopolysaccharide fraction from heart valves of adult steers. This may be attributable to differences in

Fig. 2. Hourly incorporation of labelled glucosamine into bovine heart valve. All incubations were carried out with approx. 1 g of mixed valves in 10 ml of Krebs–Ringer bicarbonate solution containing 50 μ Ci of [3 H]glucosamine, for indicated time-intervals at 37°C under $O_2 + CO_2$ (95:5). Each point represents an average of two determinations.

the ages of the animals used and possibly also to the use of different experimental conditions. Recoveries of hexosamine content after separation on Sephadex G-50 were between 95 and 102%.

Characterization of peak I. The molecular weight of the material in this peak was greater than 10000 as judged from the elution pattern of chondroitin sulphate and glycopeptides from ovalbumin on the Sephadex G-50 column.

Table 2 shows the results of tests providing further information on the identification of peak I from each of the four different valves as mucopolysaccharides. The amounts of uronic acid in all four valves were relatively similar to that for hexosamine; the ratio of hexosamine/uronic acid was 0.983:1.109. Also peak I had much more galactosamine than glucosamine; the galactosamine/glucosamine ratio was 1.138:1.427. In this peak, the sialic acid content was negligible. Descending paper chromatography of a hydrolysed sample revealed only galactose as neutral sugar. Galactose may arise from the carbohydrate–peptide linkage region or also could indicate that a small amount of keratan sulphate is present in this tissue. On electrophoresis on cellulose acetate strips, Alcian Blue-staining bands corresponding to standard hyaluronic acid and chondroitin sulphates were respectively obtained from peak I of all valves.

Thus, from the above results, it was decided that peak I consisted of mucopolysaccharides whose main components were chondroitin sulphates and hyaluronic acid. Moreover, the bovine heart valve might have more galactosamine-containing mucopolysaccharides than glucosamine-containing mucopolysaccharides. This observation is in agreement with other studies (Deiss & Leon, 1955; Meyer *et al.* 1956; Moretti & Whitehouse, 1963).

Characterization of peak II. To obtain analytical evidence that peak II from each of the four valves indeed consisted of glycopeptides, further analysis for the peptide and the carbohydrate portion were carried out. Samples were analysed for uronic acid, neutral sugars, fucose, glucosamine, galactosamine

Table 1. Distribution of hexosamine of bovine heart valves in two peaks in eluates from a Sephadex G-50 column

Eluate fractions corresponding to peaks I and II (see Fig. 1) were combined, dialysed, concentrated and analysed for hexosamine. The assay conditions are described in the text. Each value was obtained from pooling tissue from each valve of five hearts. Percentages of hexosamine contents from each peak are given in parentheses.

Valve ...	Concn. of hexosamine (μ mol/100 mg of dry fat-free tissue)			
	Aortic	Pulmonary	Mitral	Tricuspid
Peak I	7.49 (81.8)	5.90 (76.3)	7.67 (80.1)	6.44 (77.8)
Peak II	1.67 (18.2)	1.83 (23.7)	1.90 (19.9)	1.84 (22.2)

Table 2. *Concentration of chemical components in peak I*

This peak was prepared as noted in the legend for Table 1. The combined eluates of peak I were assayed for uronic acid and sialic acid, and also for separation of hexosamines, by using a paper-chromatographic system. The details of the separation of glucosamine and galactosamine and their quantitation, as well as the assays for uronic acid and sialic acid, are given in the text. Each value was obtained for pooled tissue from each valve of five hearts.

Component	Valve ...	Concentration ($\mu\text{mol}/100\text{ mg}$ of dry fat-free tissue)			
		Aortic	Pulmonary	Mitral	Tricuspid
Uronic acid		7.18	5.32	7.80	6.32
Glucosamine		3.09	2.76	3.16	2.62
Galactosamine		4.40	3.14	4.51	3.82
Sialic acid		0	0	0	0
Ratio					
Hexosamine/uronic acid		1.043	1.109	0.983	1.019
Galactosamine/glucosamine		1.424	1.138	1.427	1.458

Table 3. *Composition of chemical components in peak II*

Peak II was prepared as noted in the legend for Table 1. This peak was used for the separation of the hexosamines and also for the determinations of uronic acid, neutral sugars, fucose and sialic acid. The conditions of the various assays are given in the text. Each value was obtained for pooled tissue from each valve of the five hearts. Galactosamine/glucosamine ratios obtained from a column-chromatographic technique for amino acid analysis, are given in parentheses. Amino acid analyses were done on pulmonary and mitral valves.

Component	Valve ...	Concentration ($\mu\text{mol}/100\text{ mg}$ of dry fat-free tissue)			
		Aortic	Pulmonary	Mitral	Tricuspid
Uronic acid		0	0	0	0
Neutral sugars		3.12	3.27	3.27	3.63
Fucose		0.21	0.23	0.24	0.25
Glucosamine		1.18	1.33	1.30	1.21
Galactosamine		0.49	0.50	0.60	0.63
Sialic acid		0.79	0.88	0.88	0.95
Aspartic acid			0.65	0.58	
Threonine			0.27	0.29	
Serine			0.20	0.21	
Glutamic acid			0.48	0.46	
Proline			0.35	0.34	
Glycine			0.42	0.44	
Ratio					
Galactosamine/glucosamine		0.415	0.376 (0.353)	0.461 (0.399)	0.521
Sialic acid/hexosamine		0.473	0.481	0.463	0.516
Hexosamine/neutral sugars		0.535	0.560	0.581	0.507

and sialic acid (Table 3). In contrast with peak I, there was no uronic acid present in this peak, also the amount of glucosamine was higher than that of galactosamine; the galactosamine/glucosamine ratio was 0.376:0.521. Approx. 7% of neutral sugars was measured as fucose. Ratios of sialic acid/hexosamine and of hexosamine/neutral sugars were 0.463:0.516 and 0.507:0.581, respectively.

By descending paper chromatography of neutral sugars, spots corresponding to galactose, glucose

and mannose were obtained. This result suggested that there was an appreciable amount of glucose present in peak II; it was actually present to the same extent as galactose and mannose. This finding is in agreement with the results obtained by Moczar & Moczar (1967) on calf heart-valve prepared by their urea extraction method. Thus, it may be that the presence of glucose is a striking feature of valvular glycoprotein.

For the amino acid analyses pulmonary and

mitral valves were chosen as representative of semi-lunar and atrioventricular heart valves respectively. The values obtained for the amino acids are shown in Table 3. The results obtained with both valves were very similar. Of note in these amino acid analyses is the presence of a large number of aspartic acid, glutamic acid, glycine, proline, threonine and serine residues; other amino acid residues were present in almost negligible amounts. Some of these amino acids are highly characteristic of various glycopeptides; the possibility of contamination by other peptides, arising for example from collagen, is unlikely. Incidentally, the percentages of protein calculated by adding the values obtained by amino acid analysis was approx. 23%; total sugars were approx. 77%. The values were similar for pulmonary and mitral valves.

The average molecular weight of components of this peak was 3500 as judged by gel filtration described above and this value seems to be reasonable for glycopeptide.

Sialic acid, hexosamine, fucose, galactose and glucose are also components of glycolipids. Since the materials used in this study have been defatted thoroughly with acetone and chloroform-methanol, the presence of glycolipids is not likely.

From these results, it is apparent that peak II consisted of glycopeptides.

Chemical differences among the four valves. There was some variation in the amounts of the mucopolysaccharide components among the various types of valves (see Tables 1 and 2). In general, mitral and aortic valves had higher concentrations of hexosamines and uronic acid; the pulmonary valve had a lower content of all components, whereas values for tricuspid valve were intermediate. On the basis

of the galactosamine/glucosamine ratio among the valves only the pulmonary valve appeared to have a slightly different composition of mucopolysaccharides.

Concerning the glycopeptides, there were no remarkable differences in the amounts of various components (see Tables 1 and 3). However, there were slight differences in the galactosamine/glucosamine ratios among the four valves and this might indicate that the composition of glycopeptides may not be identical in each valve, even though no differences in major molecular weight components were found by gel filtration.

Incorporation of labelled glucosamine into the two peaks. Table 4 shows the radioactivity incorporated into various amino sugars of bovine heart valves. Their distributions in the two peaks were very similar in all four valves; 77–79% in peak I, and 21–23% in peak II. The sums of peaks I plus II corresponded to 79–85% of radioactivities in the papain digests before separation on Sephadex G-50. Also, distribution of radioactivity among the several amino sugars was quite similar in each valve. Approx. 68–74% of total radioactivity in peak I was found as glucosamine and the remainder as galactosamine. In peak I, the sialic acid and its radioactivity was negligible; none was found in all four valves. On the other hand, 15–19% of total radioactivity in peak II was present as sialic acid, and radioactivities of glucosamine and galactosamine accounted for 54–61% and 22–28% respectively. It should be noted that the radioactivity in sialic acid accounted for over 15% of the total radioactivity present in the glycopeptide fraction. This shows that the pathways for the formation of sialic acid from glucosamine via

Table 4. *Distribution of radioactivity in peaks I and II from Sephadex G-50 column*

Peaks I and II were used for separation of hexosamines by a paper-chromatographic system and also for the identification of sialic acid, by using Dowex 1 (X8) column chromatography. Bray's (1960) solution was used in measuring the radioactivity of these substances. Each value was obtained for pooled tissue from each valve of five hearts. Percentages of total radioactivities from each peak are given in parentheses.

		Sp. radioactivity (d.p.m./100mg of dry fat-free tissue)			
	Valve ...	Aortic	Pulmonary	Mitral	Tricuspid
Peak I					
Glucosamine		1200 000	816 000	1 851 000	2 852 000
Galactosamine		597 000	330 000	752 000	1 008 000
Total		1 779 000 (78.6)	1 146 000 (77.3)	2 603 000 (78.3)	3 860 000 (78.3)
Peak II					
Glucosamine		286 000	206 000	415 000	572 000
Galactosamine		104 000	82 000	173 000	301 000
Sialic acid		83 000	49 000	132 000	196 000
Total		483 000 (21.4)	337 000 (22.7)	720 000 (21.7)	1 069 000 (21.7)

Table 5. *Specific radioactivity of amino sugars in the two peaks*

Specific radioactivity was obtained by calculation from data for radioactivities of the amino sugars in Table 4 and the amounts of amino sugars in Tables 2 and 3.

	Valve ...	Sp. radioactivity (d.p.m./ μ mol of amino sugar)			
		Aortic	Pulmonary	Mitral	Tricuspid
Peak I					
Glucosamine		388 000	296 000	586 000	1 089 000
Galactosamine		131 000	105 000	167 000	264 000
Peak II					
Glucosamine		242 000	155 000	319 000	473 000
Galactosamine		212 000	164 000	289 000	478 000
Sialic acid		118 000	56 000	150 000	206 000

UDP-*N*-acetylglucosamine are quite active in the connective-tissue cells of the bovine heart valve.

However, there were remarkable differences in the pattern of incorporation into the components of the various valves. The absolute values of all components of both peaks were found to be increased in the following order without exception: pulmonary, aortic, mitral and tricuspid valves.

Specific radioactivities of amino sugars. Table 5 shows the specific radioactivities of various amino sugars of both peaks. The specific radioactivity of glucosamine was higher than that of galactosamine in peak I. These findings suggest that glucosamine-containing mucopolysaccharides have a more rapid rate of turnover compared with galactosamine-containing mucopolysaccharide. This seems to be supported by the finding of Schiller, Mathews, Cifonelli & Dorfman (1956), who indicated that the turnover of hyaluronic acid was much faster than that of sulphated mucopolysaccharides in young rat skin. Similar results were obtained by Hardingham & Phelps (1968) with rabbit skin. On the other hand, in peak II, the values of both hexosamines were almost equal to one another in all four valves. However, the specific radioactivity of sialic acid, in all cases, was less than half that of the hexosamines in peak II. Also for all valves, the specific radioactivity of glucosamine was higher in peak I than in peak II, and the value of galactosamine was lower in peak I than in peak II.

In comparing the various valves, the order of the specific radioactivities in all components of both peaks was quite similar to the order found for total incorporations, and also to that of the absolute radioactivities of the individual amino sugars (see Table 4).

Of the two semilunar valves the aortic had a higher biosynthetic activity in the mucopolysaccharide fraction than the pulmonary valve. Similar results were obtained by Boström *et al.* (1963) in their studies of sodium [35 S]sulphate incorporation

into bovine heart valve. However, the finding in this experiment that the atrioventricular (mitral and tricuspid) valves had a higher biosynthetic activity in comparison with the semilunar valves differs from their results. In addition, a study by Hallgrímsson, Friberg & Burke (1970) with a radioautographic method showed that there was no significant difference between the four heart valves of guinea pigs with regard to turnover of the sulphated mucopolysaccharides. No explanation of such a discrepancy is apparent, though an exact comparison of the experiments of the other workers with the studies reported here is not possible, because of the different experimental conditions.

There is an apparent discrepancy in the rate of turnover of the carbohydrate moieties in glycoproteins and mucopolysaccharides in their studies, as compared with previous work reported by Kanke *et al.* (1970). This may be due to the fact that the valves of older animals were used in the present studies, whereas young calves were utilized in the earlier work. Although the hexosamine content of the valves is known to diminish with age, this diminution is more pronounced for the glycopeptides. Also in the earlier experiment (Kanke *et al.* 1970) the papain digests were divided into three fractions rather than two and the hexosamines were not separated into glucosamine and galactosamine; thus the two experiments are not directly comparable.

Of course, any definitive conclusions regarding turnover of mucopolysaccharides and glycopeptides would require a different type of experiment. It is apparent at least that glucosamine in mucopolysaccharides has the most rapid turnover, followed in turn by the turnover rates of both glucosamine and galactosamine in glycopeptides, and then that of galactosamine in mucopolysaccharides.

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