Proceedings of the
NATIONAL ACADEMY OF SCIENCES

Volume 41 · Number 5 · May 15, 1955

*THE ENZYMATIC SYNTHESIS IN VITRO OF HYALURONIC ACID CHAINS**

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Communicated by Carl F. Cori, March 18, 1955

INTRODUCTION

The structure of hyaluronic acid has been elucidated largely by the work of Meyer and collaborators,[1, 2, 3] who have shown it to be a linear polymer of alternating units of N-acetyl-D-glucosamine and D-glucuronic acid. The monosaccharide components appear to be combined in alternating β-1,3- and β-1,4-linkages, the former being those involving C_1 of GA[4] and the latter those involving C_1 of AG. Hyaluronic acid isolated from different tissues appears to have constant chemical composition[3, 5, 6] but varies in average molecular weight from 300,000 to 1,500,000.[7] No enzymatic synthesis of hyaluronic acid by cell-free preparations has been reported.

We have now been able to demonstrate the synthesis of oligosaccharide chains having the structure of hyaluronic acid by using cell-free homogenates of the Rous chicken sarcoma,[8] a tissue which has been known for a long time to produce large quantities of the mucopolysaccharide[6, 9, 10] and to have very little if any active hyaluronidase.[10] Synthesis of the oligosaccharides has been shown by incubating sarcoma homogenates with uridine diphospho-N-acetyl-glucosamine labeled with C^{14} in the N-acetyl group, together with uridine diphosphoglucuronic acid or with uridine diphosphoglucose and diphosphopyridine nucleotide. In some experiments the homogenates were incubated with uridine triphosphate and N-acetyl-glucosamine-6-phosphate labeled with C^{14} in the N-acetyl group. The preparation of the latter material with a specific yeast N-acetylase has been described.[11] UDPAg was discovered by Leloir and co-workers,[12, 13] but no coenzymatic function for it was demonstrated. UDPGA has been isolated and found to be the substrate for glucuronide synthesis by liver extracts.[14, 15] The formation of UDPGA by enzymatic oxidation of UDPG in the presence of DPN has been described by Kalckar and coworkers.[16] Evidence discussed below indicates that UDPAg and UDPGA are the substrates from which hyaluronic acid chains are synthesized.

MATERIALS AND METHODS

The C^{14}-UDPAg used was made enzymatically by incubating C^{14}-AG-6-P with UTP and a small amount of α-glucose-1,6-diphosphate[17] at pH 7.0 and 30° in the presence of Mg^{++} and an enzyme prepared by sonic disintegration[18] of baker's yeast (Fleishchmann), followed by protamine precipitation of nucleic acid and dialysis for 24 hours at 0° against 0.002 *M* EDTA, pH 6.7. The enzyme preparation contains the mutase, first studied by Leloir and Cardini,[19] which equilibrates AG-6-P and AG-1-P. We have confirmed their finding that its activity is stimulated by α-glucose-1,6-diphosphate. In addition to this mutase, our yeast extract contains the enzyme which synthesizes UDPAg by the following reaction, proceeding from left to right:

$$\text{AG-1-P} + \text{UTP} = \text{UDPAg} + \text{Inorg. pyrophosphate.} \quad (1)$$

The reversal of this reaction was first described by Smith and Munch-Petersen,[20] who found that UDPAg is pyrophosphorylated by an enzyme from rat liver nuclei to yield UTP and an unidentified second product. In the yeast extract which we have used, a stoichiometric yield of UDPAg from AG-6-P is obtained because of the presence of an inorganic pyrophosphatase which causes reaction (1) above to proceed to completion toward the right. Synthesis of C^{14}-UDPAg from C^{14}-AG-6-P has also been observed in the Rous sarcoma homogenates, where accumulation of the nucleotide has been found to occur under a variety of incubation conditions. UDPAg was identified following chromatography on Dowex-1 (formate) as a uridine derivative (ultraviolet spectrum) having one mole of acid-labile (10 minutes, pH 2, 100°) AG (modified Morgan and Elson test[21]). It migrated on a paper chromatogram (Whatman No. 1) in the neutral ammonium acetate–ethanol solvent of Paladini and Leloir[12] as a single spot, with $R_{\text{UMP-5}'} = 1.53$. Nonisotopic UDPAg was obtained from Sigma Chemical Company.

The UDPGA used was prepared enzymatically from UDPG (isolated from yeast or supplied by Sigma Chemical Company) by incubating it with DPN, according to Strominger *et al.*,[16] using an enzyme obtained by ammonium sulfate fractionation of an extract of pigeon liver acetone powder. It has been found that the sarcoma homogenates prepared as described below also contain the enzyme which oxidizes UDPG to UDPGA. The latter material has been isolated from incubation mixtures of the sarcoma homogenates by chromatography on Dowex-1 (formate) and identified subsequently by paper chromatography (as above) as a single spot having $R_{\text{UMP-5}'} = 0.76$. The material gave a glucuronic acid test according to Dische[22] and was active in the enzymatic glucuronide synthesis system of Dutton and Storey.[14]

Hyaluronic acid was isolated following addition of carrier (bovine vitreous humor HA, Worthington Biochemical Corporation) by a slight modification of the method of Harris, Malmgren, and Sylvén.[6] The resulting HA sedimented slowly in 0.04 *M* maleate buffer, pH 6.7, in the Spinco ultracentrifuge, showing a single peak with $S_{20} = 0.78$. The HA isolated from the Rous sarcoma has been reported to have an S of 1.0.[6] Hyaluronic acid was determined quantitatively as GA, using the method of Dische.[22]

Sarcomas were developed in the wing muscle of 6–8-week-old New Hampshire Red or White chickens. After 3 weeks of growth of the tumor, the chickens were

anesthetized with nembutal and bled from the neck, and the sarcomas were excised. They weighed 8–10 gm. each. Homogenates were prepared by cutting the sarcoma into small pieces with a scalpel and grinding the pieces in the Waring Blendor with twice their weight of pH 8.0 buffer (0.02 *M* THM–0.001 *M* EDTA–0.01 *M* $MgCl_2$) at 0° for four $^3/_4$-minute periods, with cooling in ice between. The mixture was centrifuged for 5 minutes at 10,000 × g, and the supernatant fluid was used.

EXPERIMENTAL RESULTS

Table 1 shows the details of experiments in which incorporation of C^{14} into material isolated with carrier HA is shown. Incorporation was obtained either from UDPAg or AG-6-P (experiments 6A and 6B). In experiment 6C the only source of uridine nucleotides was the tissue homogenate itself. Here incorporation of the isotope was not less than that observed in the experiments with added nucleotides. This finding raises the question of whether the pathway of incorporation of AG-6-P is via UDPAg. As is shown below, synthesis of C^{14}-HA is strictly dependent upon the presence of uridine nucleotides. Experiments 2A and 7 show that no incorporation of C^{14} is observed when enzymatic action is stopped at zero time by addition of ethanol. The HA from experiments 2A and 2B was dialyzed before assay. That from the other experiments was not. It has been shown that dialysis for 2 days at 5° against a large volume of water of any of the C^{14}-HA samples of Table 1 leads to a loss of from 50 to 70 per cent of the C^{14}, while only 10 to 20 per cent of the sample is lost. Electrodialysis for 48 hours at 800 volts in Visking casing removed almost all the isotope from any sample. The dialyzability of the label originally suggested that the C^{14} observed in the HA was due solely to

TABLE 1

INCORPORATION OF C^{14}-URIDINE DIPHOSPHO-N-ACETYL-GLUCOSAMINE OR C^{14}-N-ACETYL-GLUCOSAMINE-6-PHOSPHATE INTO HYALURONIC ACID BY HOMOGENATES OF ROUS CHICKEN SARCOMA

EXP. No.	URIDINE NUCLEOTIDE ADDED (μMOLES)				C^{14}-AG-6-P* (μMOLES)	INCUBATION TIME (MIN)	HA ADDED BEFORE INCUBATION (MG)	CARRIER HA ADDED AFTER INCUBATION (MG)	HA ISOLATED (MG)	SP. ACT. OF HA (COUNTS/MIN/MG GA)	TOTAL C^{14} IN HA (COUNTS/MIN)
	UDP	UTP	UDPG	UDPGA	C^{14}-UDPAg*						
6A†	12	Trace	3.27	1.0	2.44	...	90	7.5	38.5	32.2	58.7	969
6B†	2.0	20.0	3.27	...	...	4.67	90	7.5	38.5	31.6	44.4	719
6C†	...	...	...	...	...	2.33	90	3.8	19.2	21.3	47.5	517
2A‡	...	...	...	...	...	2.33	0	28.5	0	15.1	0.0	0
2B‡	0.6	50.0	0.6	...	...	3.11	120	3.4	20.0	17.3	44.5	394
7	...	...	...	...	...	2.33	0	30.0	0	26.2	1.5	20

* Compound had 67,500 counts/min/μmole. C^{14} present in the carboxyl C of acetyl group. All samples counted in a windowless flow counter, following plating in gelatin.

† Reaction mixture (8 ml. in experiments 6A and 6B) contained DPN, 1.8×10^{-3} *M*; $MgCl_2$, 5×10^{-3} *M*; THM, 1×10^{-2} *M*; EDTA, 6×10^{-4} *M*; glutathione, 12 mg. In addition, experiments 6B and 6C contained 1.2×10^{-3} *M* α-glucose-1,6-diphosphate. In experiments 6A and 6B, enzyme: 6 ml. homogenate from 2 gm. tissue. In experiment 6C (4.5 ml.), enzyme: 3 ml. homogenate from 1 gm. tissue. pH maintained at 6.8–7.5. 37°.

‡ Reaction mixture (7.1 ml.) contained glutathione, $MgCl_2$, THM, and EDTA, as in experiment 6A. In addition, experiment 2B contained DPN, 1.1×10^{-3} *M*. Enzyme: 3.5 ml. homogenate from 0.9 gm. tissue. 37°.

contamination by one or more of the labeled compounds introduced or to labeled degradation products derived from them. This possibility was examined by reprecipitating a number of samples of C^{14}-HA from large molar excesses of all substances whose presence seemed likely. Table 2 shows the results of these experiments. The recovery of HA varied from 75 to 95 per cent, and the samples retained approximately 40 per cent of their C^{14} content. Although the loss of C^{14} was about twice that of the HA, the results rule out the possibility of significant contamination of the final products by any of the substances present during the reprecipitations. The finding of a loss of C^{14} greater than that of HA on reprecipitation is explicable on the basis of the presence of the label predominantly in oligosaccharides of lower molecular weight than the carrier HA. These oligosaccharides may remain partially in the supernatant fluid each time a reprecipitation is done.

TABLE 2

RECOVERY OF C^{14}-HYALURONIC ACID FOLLOWING REPRECIPITATION UNDER VARIOUS CONDITIONS

Exp. No.	Sp. Act. of HA Taken (Counts/Min/Mg GA)	Total HA Taken As GA (Mg)	Total HA Taken As C^{14} (Counts/Min)	Calc. Dilution of C^{14} if HA Were Contaminated by C^{14}-labeled Substances Below: Mixture from Which HA Precipitated*		Mixture from Which HA Precipitated†		Recovery of HA Taken As GA (Per Cent)	Recovery of HA Taken As C^{14} (Per Cent)	Sp. Act. of HA (Counts/Min/Mg GA)
6A	58.7	1.83	107	UDPAg	1.6×10^4	.....		79.8	35.3	25.9
				AG-6-P	1.9×10^2					
6A	58.7	1.83	107	.....		Acetate	6.2×10^3	95.1	38.1	23.4
						AG	1.6×10^3			
						Tetramer	3.8×10^2			
						Hexamer	1.9×10^2			
						Octamer	*ca.* 60			
6A	58.7	6.40	376	.....		Acetate	3.6×10^3	...	...	29.5
						AG	1.8×10^2			
						UDPAg	7.2×10			
						AG-6-P	3.6×10^2			
6A‡	29.5	0.81	24	.....		Tetramer	2.9×10^3	...	77.5	...
						Dimer	4.2×10^2			
6B	44.4	2.32	102	UDPAg	1.6×10^4	.....		89.6	70.2	34.5
				AG-6-P	7.1×10^4					
6C	47.5	2.18	103	AG-6-P	7.1×10^4	.....		76.1	42.3	26.2
8A	24.5	1.67	41	.....		H_2O only		105	50.0	11.6

* Precipitation made twice with 66 per cent ethanol and 0.3 per cent NaCl from neutral aqueous solution to give the calculated over-all dilution shown.

† Precipitation made once as above. "Octamer," "hexamer," "tetramer," and "dimer" refer to oligosaccharides present in a testicular hyaluronidase digest of HA.

‡ The HA taken for reprecipitation was that isolated in the presence of acetate, AG, UDPAg, and AG-6-P.

Experiment 8A of Table 2 shows that reprecipitation of the C^{14}-HA from water alone leads to a considerable loss of isotope with little loss of HA. The dialyzability of the C^{14}-labeled substances in the HA is in accord with these observations. Table 2 shows that the oligosaccharides synthesized must be larger than the octasaccharide, inasmuch as precipitation of the C^{14}-HA in the presence of large amounts of testicular hyaluronidase digest of unlabeled HA does not reduce the C^{14} content of the samples to any greater extent than any other kind of reprecipitation. The oligosaccharides in the hyaluronidase digest were chromatographed on paper in the butanol–acetic acid–water solvent (50:15:35) used by Meyer.[2] Their identity was established by their R_f values.

We have subjected one reprecipitated sample of C^{14}-HA to exhaustive digestion by testicular hyaluronidase, as shown in Table 3. The enzymatic digest was spotted multiply on a large sheet of Whatman No. 1 paper, together with several

spots of the undigested C^{14}-HA. After development with the butanol–acetic acid–water solvent, one strip of the undigested HA and one strip of the digested sample were sprayed with the Morgan and Elson type spray described by Partridge[23] to locate the regions of the oligosaccharides. There were no colored zones on the strip of the undigested HA. Regions corresponding to the disaccharide, tetrasaccharide, and hexasaccharide were found on the strip of the digested HA. Water eluates were made separately of the starting spots and of the lower areas of the sheet which corresponded to the location of the oligosaccharides as revealed by the sprayed strips. The eluates were plated and counted. Table 3 shows the results. In the case of the undigested HA, C^{14} was found only in the starting spot. Seventy per cent of the counts were recovered from this region. After hyaluronidase treatment, no C^{14} remained at the starting line; 58 per cent of the counts were recovered as the tetrasaccharide and 7 per cent as the disaccharide. Failure to recover all the radioactivity applied to the paper probably is due to the very small amounts of material present. The results of this experiment indicate that the oligosaccharides synthesized by the sarcoma homogenates have the structure of HA.

TABLE 3

PAPER-CHROMATOGRAPHIC ANALYSIS OF PRODUCTS FROM THE ACTION OF HYALURONIDASE ON C^{14}-HYALURONIC ACID*

			Recovery of C^{14} in Eluates of Areas of Paper Indicated						
			Starting Spot		Between Tetrasaccharide and Starting Spot	Tetrasaccharide		Disaccharide	
Material Spotted on Paper	Sp. Act. of HA or HA Digest (Counts/Min/Mg GA)	Total C^{14} Spotted (Counts/Min)	Total C^{14} (Counts/Min)	Sp. Act. (Counts/Min/Mg GA)	(Counts/Min)	Total C^{14} (Counts/Min)	Sp. Act. (Counts/Min/Mg GA)	Total C^{14} (Counts/Min)	Sp. Act. (Counts/Min/Mg GA)
Undigested C^{14}-HA	29.5	33.8	23.4	31.2	0	0	0	0	0
Digested C^{14}-HA	25.2	38.3	0	0	0	22.4	41.1	2.8†	36.4

* The C^{14}-HA was the once-reprecipitated 6A of Table 2 (line 3). 7.4 mg. of HA and 2 mg. of crystalline plasma albumin were dissolved in 1.5 ml. of 0.10 *M* acetate buffer, pH 4.7, containing 0.08 per cent NaCl. Testicular hyaluronidase (1,000 turbidity reducing units, Worthington Biochemical Corporation) was added. Incubated under toluene at 37° for 24 hrs. Solution heated at 100° for 10 minutes. Chromatogram prepared as described in text.

† The accuracy of this determination is low.

Some experiments have been done which shed light on the pathway of incorporation of C^{14}-AG-6-P into HA. The incorporation observed in the absence of added uridine nucleotides (experiment 6C, Table 1) can be abolished by pretreatment of the sarcoma homogenate at pH 8.0 and 0° with one-third of its volume of wet, thoroughly washed Dowex-1 (formate), 8 per cent cross-linked. The Dowex treatment was done twice with equal portions of the resin in bulk. Such treated homogenates incorporate C^{14}-AG-6-P in the presence of UDPG, DPN, and UTP but do not yield C^{14}-HA when UTP is omitted. When C^{14}-UDPAg is added as the only isotopically labeled substance, formation of C^{14}-HA occurs with the Dowex-treated homogenate to which UDPGA or UDPG and DPN as a source of UDPGA[16] have been added. Table 4 shows the results of several experiments of this kind. The homogenates do not appear to contain an active uridyl transferase which might mediate the following reaction:

$$\text{UDPG} + \text{AG-1-P} = \text{UDPAg} + \alpha\text{-Glucose-1-phosphate.} \qquad (2)$$

An enzyme of this type has been reported by Kalckar and coworkers to occur in extracts of *S. fragilis*, where uridine diphosphogalactose was found to be formed by a direct exchange between UDPG and α-galactose-1-phosphate.[24] Experiment 9C shows that the synthesis of HA does not occur directly from AG-1-P and, therefore, is not analogous to the synthesis of glycogen from α-glucose-1-phosphate. Experiment 9B shows that AG-1-P must first be "activated" by conversion to UDPAg according to equation (1) above, before the hexosamine unit can be incorporated enzymatically into HA. UDPAg was demonstrated chromatographically in the reaction mixture from experiment 9B. Experiment 9A, in which a large amount of unlabeled acetate was added as a "trap," shows that the incorporation of C^{14} into HA from UDPAg labeled with the isotope in the acetyl group does not proceed via acetate ion, despite the presence in the homogenates of an active deacetylase for AG-6-P or AG. Dorfman and coworkers have recently found that

TABLE 4

REQUIREMENT FOR URIDINE DIPHOSPHO-N-ACETYL-GLUCOSAMINE IN HYALURONIC ACID SYNTHESIS

Expt. No.	Treatment of Homogenate	Nucleotide Added (μmoles) UTP	UDPG	UDPGA	C^{14}-UDPAg*	C^{14}-AG-6-P* Added (μmoles)	C^{14}-HA Recovered (Mg.)†	Sp. Act. of C^{14}-HA (Counts/Min/Mg GA)	Reprecipitation of C^{14}-HA‡ HA Recovered (Per Cent)	C^{14} Recovered (Per Cent)
8A§	None	Trace	2.5	0.1	1.95	...	19.5	24.5	96	57
8C§	+Dowex-1	Trace	2.5	0.1	1.95	...	||	23.4	93	70
8D§	+Dowex-1	...	...	...	...	3.11	19.2	0.0	..	...
9A§	+Dowex-1	Trace	2.5	...	1.95	...	27.7	14.2	73	81
9B§	+Dowex-1	25.	2.5	...	...	3.11	24.8	10.6	90	38
9C§	+Dowex-1	...	2.5	...	...	3.11	25.6	3.7#	91	15#
9D§	+Dowex-1	...	...	...	...	3.11	29.4	3.1#	74	0

* Compound labeled with C^{14} in the carboxyl C of acetyl group. See Table 1.

† Total HA added to reaction mixture = 35.2 mg.

‡ In experiment 8, 3.2 mg., and in experiment 9, 5.5 mg., of C^{14}-HA reprecipitated with 66 per cent ethanol and 0.3 per cent NaCl in presence of 10 μmoles Na acetate, 0.2 μmole UDPAg, 2.3 μmoles AG, 0.58 μmole AG-6-P, 0.075 μmole N-acetyl-hyalobiuronic acid ("dimer"), and 0.52 μmole "tetramer."

§ Reaction mixture (7.8 ml.) contained: DPN, 2×10^{-3} *M*; $MgCl_2$, 4×10^{-3} *M*; THM, 8×10^{-3} *M*; EDTA, 4×10^{-4} *M*; glutathione, 10 mg. Enzyme: 4.5 ml. from 1.5 gm. tissue. 37°. 90 minutes. In experiments 8A and 8C, UDP, 6.4×10^{-4} *M*. In experiment 9A, Na acetate, 4.3×10^{-2} *M*. In experiments 9B, 9C, and 9D, α-glucose-1,6-diphosphate, 7×10^{-6} *M*. Experiment 8 maintained at pH 7.3–7.5. Experiment 9 maintained at pH 7.0–7.3.

|| No estimate available of total HA recovery since part of preparation was lost.

The accuracy of this determination is low.

the incorporation of AG labeled with C^{14} in the acetyl group into HA by whole cells of group A hemolytic streptococci could be attributed to its prior deacetylation to C^{14}-acetate.[25] This organism apparently possesses an active deacetylase for AG and was found to incorporate C^{14}-labeled glucosamine into HA. The authors discussed the possibility that HA synthesis proceeds from AG via acetate ion, which reacts with an unknown substance to form a precursor of the mucopolysaccharide. On the basis of what is now known about glucosamine metabolism, it seems likely that the unknown substance in their system was really Gm-6-P, which is acetylated by acetyl-coenzyme A, as has now been described.[11] The AG-6-P formed is incorporated into HA first by conversion to AG-1-P and then to UDPAg. It is known that Gm-6-P is formed by the action of yeast hexokinase on glucosamine.[26] The incorporation of C^{14}-glucosamine observed by Dorfman and coworkers was most likely due to prior phosphorylation of the free sugar by the hexokinase of the cells, one of the possibilities which they mentioned. Leloir and Cardini have reported that Gm-6-P is formed enzymatically in extracts of

Neurospora crassa by a kind of transamination from glutamine to hexose-6-phosphate.[19] We have found the N-acetylase for Gm-6-P to be active in the sarcoma homogenates. We have not yet tested the homogenates for the presence of the enzyme system described by Leloir and Cardini by which the Gm-6-P required for HA synthesis may be produced ultimately from blood glucose. Dorfman and coworkers have already shown that the carbon skeletons of the glucosamine and glucuronic acid units of streptococcal hyaluronic acid are derived from glucose without prior scission of the carbon chain.[27]

DISCUSSION

The work reported here shows that HA synthesis occurs enzymatically either by transfer of AG (and, presumably, of GA) residues from UDPAg (and UDPGA) to the terminal monosaccharide units of pre-existing chains or by preliminary reaction of these two uridine-linked sugars to form a uridine-linked disaccharide, which then adds its disaccharide unit to a pre-existing chain or to another uridine-linked oligosaccharide. Under the conditions we have studied, relatively short-chain oligosaccharides (M.W., > 2,000) apparently become the predominantly labeled species. The detailed mechanism and degree of reversibility of these synthetic reactions are being studied.

SUMMARY

1. Cell-free homogenates of the Rous chicken sarcoma have been found to synthesize oligosaccharide chains composed of N-acetyl-D-glucosamine and D-glucuronic acid units combined as in hyaluronic acid, by incubating them with C^{14}-N-acetyl-D-glucosamine-6-phosphate and uridine triphosphate or with C^{14}-uridine diphospho-N-acetyl-D-glucosamine. In either case, the source of glucuronic acid units was presumably uridine diphospho-D-glucuronic acid, formed by enzymatic oxidation of added uridine diphosphoglucose in the presence of diphosphopyridine nucleotide.

2. The C^{14} label has been shown to be a part of the oligosaccharide structure by reisolation of the C^{14}-hyaluronic acid from large molar excesses of all substances, added in nonisotopic form, which might have been present as C^{14}-labeled contaminants in it.

3. Presence of the C^{14} label in the oligosaccharides themselves has also been shown by exhaustive enzymatic digestion of the C^{14}-hyaluronic acid with testicular hyaluronidase and subsequent chromatographic isolation of a small amount of C^{14}-N-acetyl-hyalobiuronic acid and of a large amount of the corresponding C^{14}-labeled tetrasaccharide.

4. The incorporation of isotope from C^{14}-N-acetyl-D-glucosamine-6-phosphate has been shown to occur via uridine diphospho-N-acetyl-glucosamine, which appears to be an obligatory intermediate.

5. The sarcoma homogenates have been shown to contain all the enzymes necessary to synthesize hyaluronic acid from uridine diphosphoglucose and D-glucosamine-6-phosphate. The pathway of this synthesis is discussed.

* This work was supported in part by a grant from the Nutrition Foundation, Inc.

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[4] The symbols used in the text refer to compounds as follows: AG, N-acetyl-D-glucosamine; AG-6-P, N-acetyl-glucosamine-6-phosphate; AG-1-P, N-acetylglucosamine-1-phosphate; Gm-6-P, D-glucosamine-6-phosphate; GA, D-glucuronic acid; UDPAg, uridine diphospho-N-acetyl-glucosamine; UPDGA, uridine diphosphoglucuronic acid; UDPG, uridine diphosphoglucose; UTP, uridine triphosphate; UDP, uridine diphosphate; UMP-5′, uridine-5′-phosphate; HA, hyaluronic acid; DPN, diphosphopyridine nucleotide; EDTA, ethylenediamine tetraacetic acid; THM, tris-(hydroxymethyl)-aminomethane.

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