Title	ヒト胎盤糖質の研究(第7報) : セルロース・イオン交換体による分画とキシロースの確認
Sub Title	Studies on the carbohydrates extracted from human placenta. VII. : fractionation by ion-exchange cellulose chromatography and identification of D-xylose.
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Publisher	共立薬科大学
Publication year	1965
Jtitle	共立薬科大学研究年報 (The annual report of the Kyoritsu College of Pharmacy). No.10 (1965.) ,p.5- 11
JaLC DOI	
Abstract	
Notes	
Genre	Technical Report
URL	https://koara.lib.keio.ac.jp/xoonips/modules/xoonips/detail.php?koara_id=AN00062898-00000010-0005

ヒト胎盤糖質の研究（第7報） セルロース・イオン交換体による分画とキシロースの確認

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Studies on the carbohydrates extracted from human placenta.[1] Ⅶ. Fractionation by ion-exchange cellulose chromatography and identification of D-xylose.*

Masashi Tomoda and Kimie Murayama

The glycoprotein extracted from human placenta was treated with a protease and polysaccharide fraction so obtained was fractionated by the chromatographys using DEAE-cellulose, ECTEOLA-cellulose, and CM-cellulose.

By the application of paper-chromatographic and gas-chromatopraphic techniques, the componental sugars of each fraction were examined. D-Galactose, D-glucose, D-mannose, D-xylose, L-fucose, D-glucosamine, and N-acetyl neuraminic acid were identified. They were also determined by the quantitative colorimetry.

In 1956, one of the authors reported that there was D-xylose in the component sugars of the polysaccharides extracted from human placenta.[2] This was the first report that showed the presence of D-xylose in animal tissue, and after that, few reports on D-xylose in animal body have been presented.[3][4] In these experiments, D-xylose and other sugars were only detected by papar chromatography.

The glycoprotein extracted from human placenta has now been treated with a protease, and fractionated by the chromatography on ion-exchange cellulose, and is described in the present paper. The sugar components of each fraction have been identified by means of both paper and gas chromatography, and determined by quantitative colorimetry in this work.

Materials and Methods

Extraction of glycoprotein from human placenta: This was similar to that described in the first paper[2] of this series. Fresh normal hnman placentae were washed with ice-water, cleaned of non-placental tissue and blood, and ground with acetone. This material was exhaustively extracted with acetone and dried in air.

Acetone-dried material was suspended in aqueous 30% potassium chloride containing 0.1% potassium carbonate and occasionally shaken for a week at 37°C. The liquid extract was dialyzed for 2 days against running water. To the clear liquid

* Japan. J. Exp. Med., 35巻, No. 3 (1965) に発表.

1) Part Ⅵ; M. Tomoda: Yakugaku Zasshi, **77**, 669 (1957).
2) S. Akiya, M. Tomoda: *Ibid.*, **76**, 571 (1956).
3) H. Masamune, Z. Yoshizawa, K. Tokita: Tohoku J. Exp. Med., **66**, 51, 251 (1957).
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was added acetic acid to pH 4, filtered after standing an hour, and the filtrate was evaporated *in vacno* to 1/20 volume. Equal volume of 10% trichloroacetic acid was added under ice-cooling, and the mixture was stirred and tne precitate was removed by centrifuging. The liquid was dialysed for 1 day against running water, and then evaporated *in vacuo* to dryness.

Enzymic Digestion : 5 g. of the extracted glycoprotein and 0.1 g. of pronase-P (Kaken-Kagaku Co.) were dissolved in 100 ml. of 15 mM calcium acetate solution, and the solution was incubated at 37°C. Proteolysis was followed by means of ninhydrin method,[5] reaction was completed after 20 hr. The solution was filtered and the filtrate was concentrated *in vacuo* to 20 ml., and 80 ml. of ethanol was added. The precipitate was obtained by centrifuging, and freeze-dried. This material was used as a sample for chromatography on ion-exchange cellulose.

Chromatography on DEAE-cellulose : DEAE-cellulose (Brown Co.) was purified by treatment with 0.5*N* sodium hydroxide until the washings showed no color by phenol-sulfuric acid method,[6] and after removal of the excess of sodium hydroxide by repeated washing with distilled water, was equilibrated with 0.01 *M*-acetate buffer (pH 5.0). For the separation of 0.5 g. of the sample, a DEAE-cellulose column (40× 135 mm.) was used, and fractions were collected at 20 ml. The mucopolysaccharides were eluted stepwise by increasing the ionic strength of the eluting buffer, and determined by phenol-sulfuric acid method[6] (E at 490 mμ). Salt and alkali were removed from the effluent by dialysis.

Chromatography on ECTEOLA-cellulose : ECTEOLA-cellulose (Serva Co.) was purified and used by the same manner as that of DEAE-cellulose. For the separation of 1 g. of the sample, a ECTEOLA-cellulose column (40×180 mm.) was used.

Chromatography on CM-cellulose : Like the other two ion-exchange celluloses, CM-cellulose (Brown Co.) was purified by treatment with 0.5 *N*-sodium hydroxide, distilled water, 0.5 *N*-hydrochloric acid, and again distilled water. Then it was equilibrated with 0.005 *M*-acetate buffer (pH 5.0.). For the separation of 1 g. of the sample, a CM-cellulose columm (40×285 mm.) was used.

Paper Chromatography : Polysaccharides were hydrolysed with 2 *N*-hydrochloric acid (for sialic acid, 0.1*N*-sulfuric acid at 80°C for 1hr.). Single-dimensional ascending paper chromatography was used with Tôyô-Roshi No. 51 paper and the following solvent systems : A, n-butanol : benzene : pyridine : water (5 : 1 : 3 : 3, by vol., upper layer); B, ethyl acetate : pyridine : water (10 : 4 : 3, by vol.); C, n-butyl acetate : acetic acid : ethanol : water (3 : 2 : 1 : 1, by vol.); D, water saturated collidine; E, n-butanol : n-propanol : 0.1*N*-hydrochloric acid (1 : 2 : 1, by vol.).

5) E. W. Yenom, E. C. Cocking : Biochem. J., 58, xii (1954).
6) M. Dubois, K. A. Gilles J. K. Hamilton, P. A. Rebers, F. Smith : Anal. Chem., 28, 350 (1956).
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The neutral sugars and glycerol spots were revealed with benzidine-acetic acid,[7] *p*-anisidine hydrochloride,[8] and periodate-permanganate reagent[9]; hexosamine was detected by minhydrin and Ehrlich reagent[10]; sialic acid was identified by orcinol[11] and thiobarbituric acid reagent.[12]

Gas Chromatograpy : This was carried out by the method of Yamakawa, Ueta & Ishizuka.[13] The methanolysed materials were converted to TMS-derivatives and applied to gas chromatography using a Hitachi model F6 with 5% Ucon LB 550 X on gaschrom CLH at 200 °C.

Determination of Sugars : Hexoses were determined by the anthrone method.[14] Xylose was determined by the orcinol method.[15] Fucose was estimated by the thioglycolic acid method.[16] Quantitative glucosamine analysis was made by the method of Rondle & Morgan[17] after hydrochloric acid hydrolysis. For the quantitative analysis of sialic acid, Ehrlich reagent[18] was employed.

Results

Fractionation by Chromatogrphy : Figs. 1-1 to 1-3 show the chromatograms obtained with carbohydrates on the three ion-exchange cellulose columns. Elution of the sample from a DEAE-cellulose column gave the four groups : D 1, tubes 6 ~

Fig. 1. Chromatograms on Ion-exchange Celluloses.

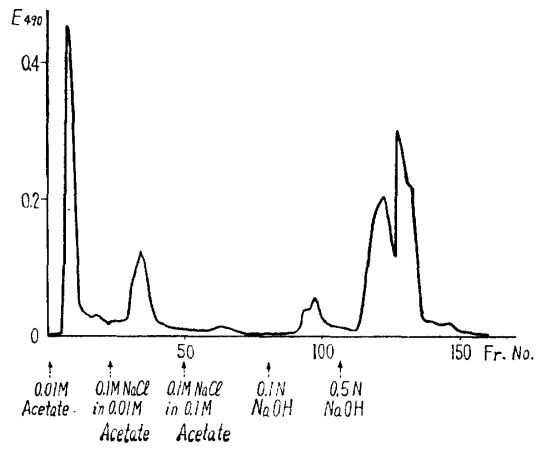

Fig. 1-1.

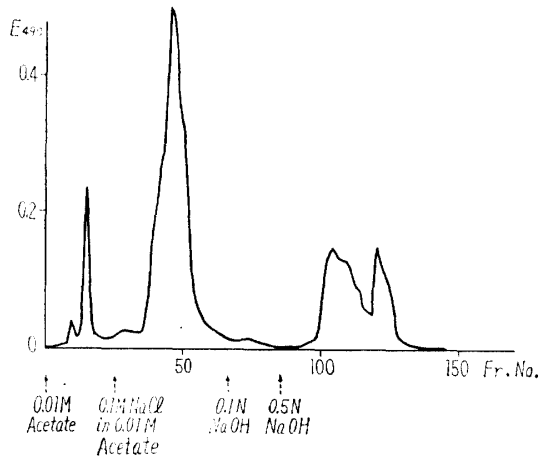

Fig. 1-2

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9) R. U. Lemieuex, H. F. Bauer : Anal. Chem., **26**, 920 (1954).
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14) R. Johanson : Anal. Chem., **26**, 1331 (1954).
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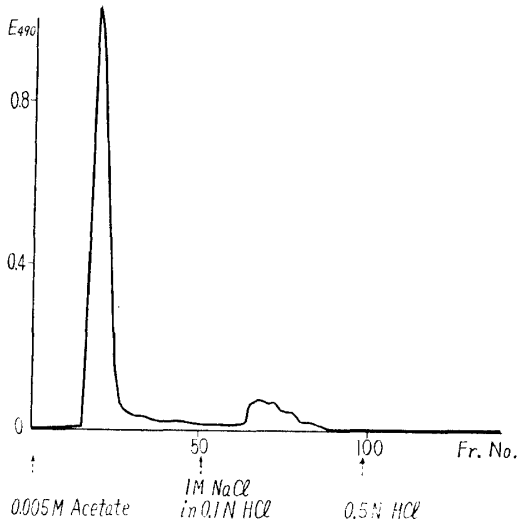

Fig. 1-3.

12; D 2, tubes 30~37; D 3, tubes 94~99; D 4, tubes 116~136. In the case of a E-CTEOLA-cellulose column, it gave the four groups : E 1, tubes 13~18; E 2, tubes 37~58; E 3, tubes 100~119; E 4, tubes 120~127. By a CM-cellulose column, it gave the two groups : C 1, tubes 16~26; C 2, tubes 65~78. The eluates obtained from tubes of each group were collected and, after mixing together and dialysis, were freeze-dried and analyzed.

Suger Components of Each Fraction : Table 1 shows the sugars detected by paper chromatography and gas chromatography. In the various solvent systems, Rf values of the revealed sugars in paper chromatography are given in Table 2. Gas-chromatograms of the neutral componental sugars of each fraction are given in Figs. 2-1 to 2-10.

Fig. 2. Gas-chromatograms of TMS-methyl glycosides.

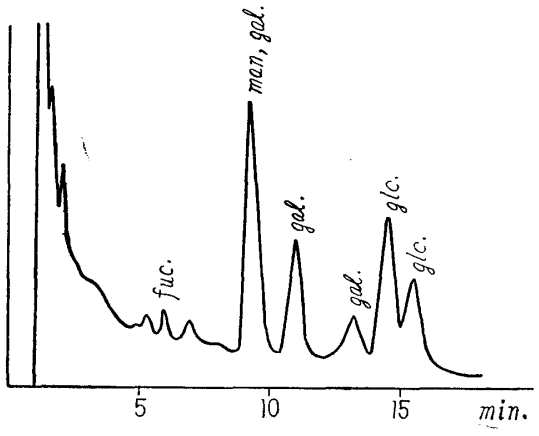

Fig. 2-1.

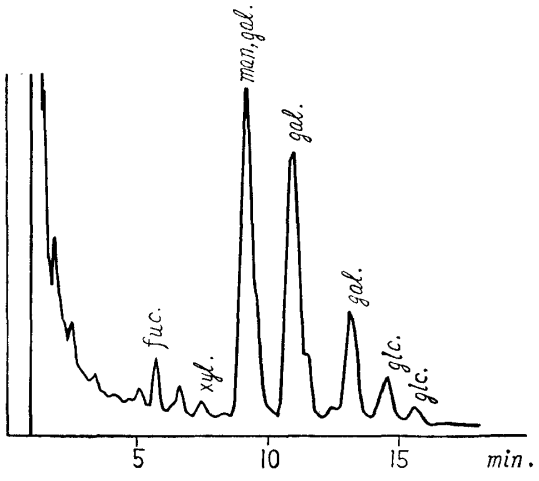

Fig. 2-2.

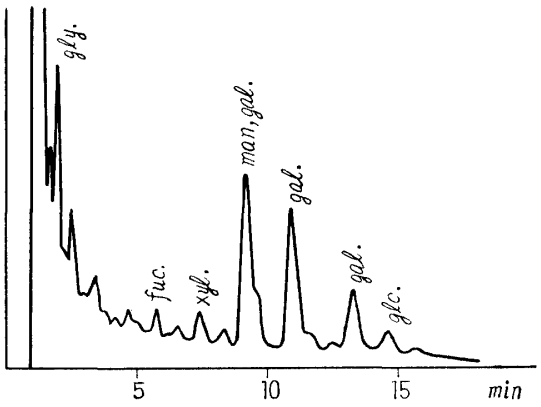

Fig. 2-3.

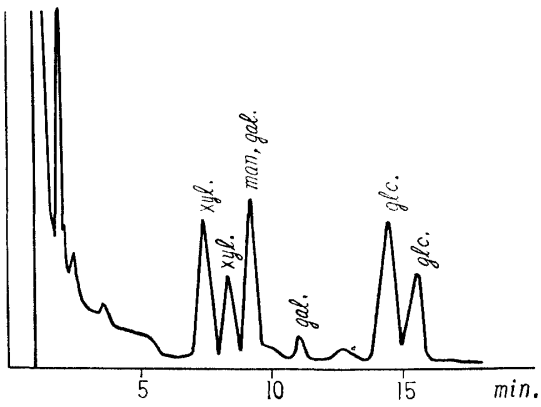

Fig. 2-4.

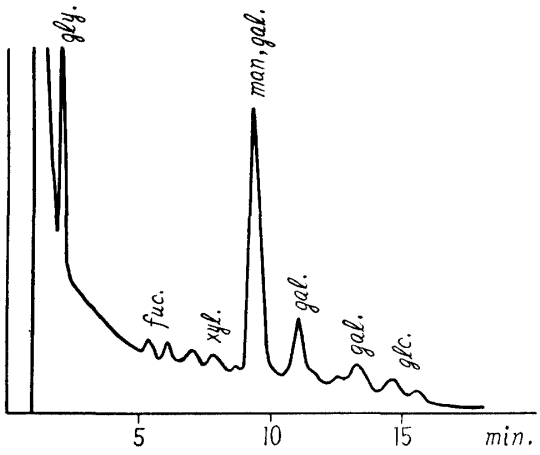

Fig. 2-5.

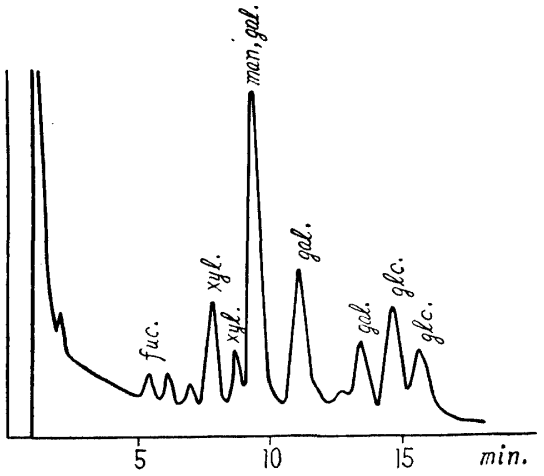

Fig. 2-6.

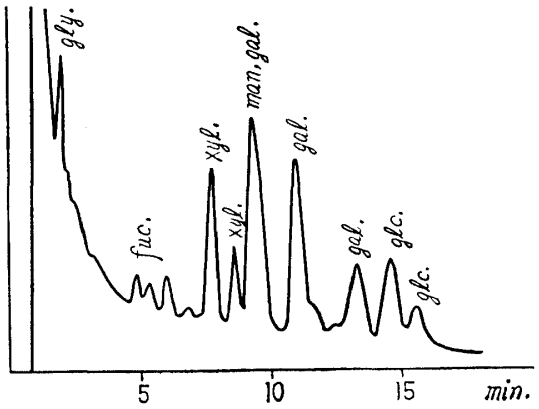

Fig. 2-7.

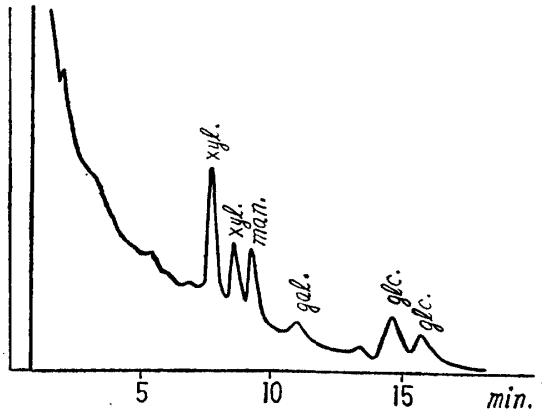

Fig. 2-8.

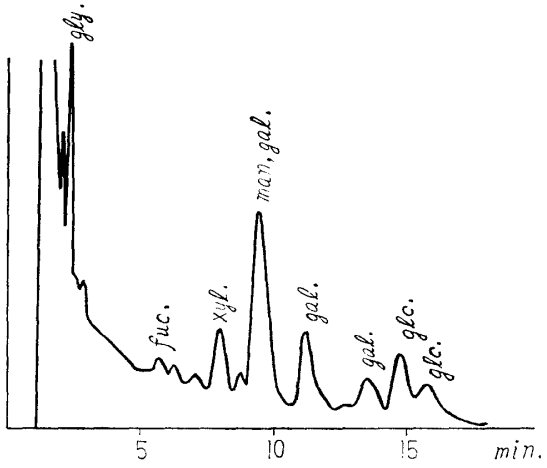

Fig. 2-9.

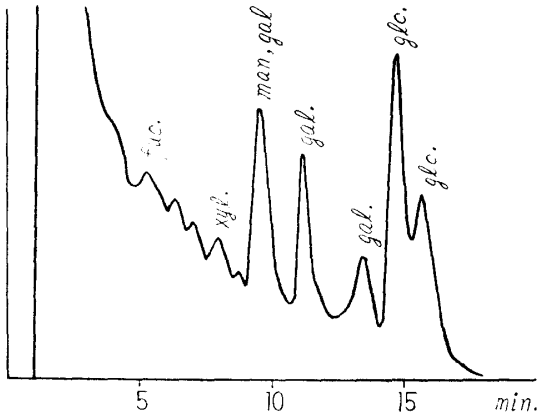

Fig. 2-10.

Table I. Sugar components of fractions

	D-Gal.	D-Glc.	D-Man.	D-Xyl.	L-Fuc.	D-$GlcNH_2$	Ac. Neu. ac.
D 1	##	##	##		+	##	##
D 2	##	##	##	+	##	##	##
D 3	##	+	+	##	+	##	##
D 4	+	##	##	##			
E 1	##	+	##	+	+	##	##
E 2	##	##	##	##	##	##	##
E 3	##	##	##	##	+	##	##
E 4	+	##	##	##		##	
C 1	##	##	##	##	##	##	##
C 2	##	##	##	+	+	##	

: distinctly detected by paper chromatography.
+ : only revealed by gas chromatography.

Table II. Rf Values of revealed sugars and glycerol in paper Chromatography.

	solvent A	solvent B	solvent C	solvent D	solvent E
L-fucose	0.40	0.59	0.35	0.56	0.41
D-xylose	0.39	0.62	0.29	0.62	0.36
D-mannose	0.33	0.54	0.23	0.59	0.32
D-glucose	0.28	0.49	0.18	0.52	0.26
D-galactose	0.23	0.42	0.17	0.46	0.23
D-glucosamine-HCl	0.19	0.33	0.10	0.37	0.20
(D-galactosamine -HCl*)	0.16	0.28	0.09	0.30	0.18
N-acetyl neu. ac.					0.37
(N-glycolyl neu. ac.*)					0.30
glycerol	0.53			0.70	0.59

* D-Galactosamine and N-glycolyl neuraminic acid were not detected in the all fractions, but the Rf values of them are noted for comparisons.

Sugar Composition and Yield of Each Fraction: The quantitative analytical data of sugars and yield of each fraction on the chromatography are shown in Table 3.

Table Ⅲ. Yields and sugar composition (%) of fractions.

fraction	yield (%)	hexose	xylose	fucose	glucosamine	sialic acid
D 1	5.4	35.3 (as gal.)		(trace)	11.6	3.2
D 2	9.4	18.2 (〃)	(trace)	0.6	10.3	3.3
D 3	2.1	5.4 (〃)	11.4	(trace)	7.8	2.9
D 4	2.7	34.1 (as glc.)	11.9			
E 1	2.4	31.0 (as gal.)	(trace)	(trace)	18.6	1.0
E 2	25.6	22.2 (〃)	2.8	0.5	13.5	5.8
E 3	5.7	15.7 (〃)	2.4	(trace)	9.0	7.0
E 4	1.1	12.6 (as glc.)	6.0		3.3	
C 1	28.2	33.5 (as gal.)	3.5	0.7	23.7	12.7
C 2	1.6	20.9 (〃)	(trace)	(trace)	5.1	

Discussion

From the results of the fractionations using the three types of ion-exchange cellulose and of the analyses on these fractions, it may be concluded that the carbohydrates in human placenta have various types. D-Galactose, D-glucose and D-mannose were found in the all fractions. D-Xylose and D-glucosamine were also widely distributed. L-Fucose and N-acetyl neuraminic acid were found in the several fractions, but the former was very small amount. The presence of galactosamine and glucuronic acid in human placental component was reported by Masamune & Yoshizaki,[19] but they have not been found in the carbohydrates obtained by authors. This difference may result from the method of extraction.

In the each fraction, C 1 gave the best result in respect of the yield and the amounts of componental sugars, and E 2 next best result. Both of them have the same seven componental sugars. D 3 and D 4 had a characteristic of high content of D-xylose. It was considered that the all fractions have the necessity of further purification from the data of electrophoresis, and the authors have purified C 1 to a few carbohydrate fractions and are making the studies on these properties. These results will be described in the next report. Glycerol was distinctly detected in the several fractions, and the question whether it is combined with the carbohydrate or not, will be solved by that time.

The authors are greatly indebted to Prof. T. Yamakawa, the Institute for Infectious Diseases, University of Tokyo, for his kindly guidance and help on the gas chromatopraphy.

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