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FRACTIONATION AND CHARACTERIZATION OF ACIDIC OLIGOSACCHARIDES AND GLYCOPEPTIDES FROM NORMAL AND PATHOLOGICAL URINES

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SUMMARY

A general procedure is described for the isolation of urinary acidic oligosaccharides and glycopeptides resulting from catabolism of glycoproteins. This procedure has been applied to normal urine and to urine from patients with diseases of the metabolism, including mucolipidosis and fucosidosis.

INTRODUCTION

In previous papers [1, 2], we described a procedure for the fractionation of urinary fucose-containing oligosaccharides, using charcoal—Celite chromatography. This procedure was also applied to the isolation of oligosaccharides accumulated in the urine of patients with Sandhoff disease [3] and mannosidosis [4]. We later modified the experimental conditions in order to study glycopeptides and acidic oligosaccharides resulting from the catabolism of glycoconjugates. We applied this procedure to cases of fucosidosis, mucolipidosis II (I-cell disease) and two new types of mucolipidosis recently described by Maroteaux and Durand (unpublished results).

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EXPERIMENTAL

Chromatographic and electrophoretic analysis

Descending paper chromatography was carried out on Whatman No. 3 paper (46 × 56 cm), using the solvent systems S1, 1-butanol—acetic acid—water (4:1:5); and S2, ethyl acetate—pyridine—acetic acid—water (5:5:1:3). Paper electrophoresis was conducted on Whatman No. 3 paper using a pyridine—water (15:1935) buffer adjusted to pH 5.4 with acetic acid, in an electric field of 10 V/cm and with a separation time of 16 h. Sugars were stained with aniline oxalate reagent (aniline—ethanol—2.5% oxalic acid aqueous solution, 2:100:150, v/v/v) and glycopeptides and amino acids with 1% ninhydrin solution in acetone.

Monosaccharide and amino acid analysis

Monosaccharides obtained after acid hydrolysis (4 *N* CF_3COOH; 100°; 4 h) were identified by paper chromatography in solvent S2. Molar ratios of monosaccharides were determined by combining colorimetric methods [5] and the gas—liquid chromatography [6] of methylglycosides obtained by methanolysis of oligosaccharides and glycopeptides. Molar ratios of hexosamines and amino acids were determined using a Beckman Multichrom Autoanalyser after acid hydrolysis (4 *N* HCl for 4—24 h at 100°).

Fractionation of acidic oligosaccharides from normal urine

Urine was collected from a 30-year-old man (blood group A Lewis b). Bacterial growth was prevented by the addition of sodium azide (1 part per 10,000). Demineralization was performed batchwise by successive treatments with a cation exchanger (Dowex 50-X8, H^+; 25—50 mesh) and an anion exchanger (Dowex 1-X8, $HCOO^-$; 25—50 mesh) (approximately 300 g of resin per litre of urine). After filtration, the effluents corresponding to 20 l of urine were pooled and concentrated to 2 l under vacuum at 35°. This solution was submitted to adsorption chromatography using a 50 × 50 cm column of charcoal—Celite prepared according to Whistler and Durso [7]. Trace amounts of hydrochloric acid, used for deactivation of charcoal, were eliminated before packing the column by ten successive washings with water (under vacuum) and dryings at 40°. Monosaccharides were eluted from the column with 6 l of water and oligosaccharides and glycopeptides were desorbed with 4 l of ethanolic solutions, the concentration of which varied discontinuously from 3.5 to 50% (ethanol—water) (see Fig. 2). Each eluted fraction was dried under vacuum, the residues were dissolved in 50—100 ml of water, and the resulting solutions were chromatographed on two parallel columns of ion exchanger (20 × 2 cm Dowex 50-X2, H^+, 200—400 mesh; 20 × 2 cm Dowex 1-X2, CH_3COO^-, 200—400 mesh). After washing with 300 ml of water, the columns were separated and the anionic exchanger was eluted with a discontinuous gradient of pyridine acetate (pH 5.4) varying from 1 to 500 m*M* (see Fig. 4). One litre of each solution was directly collected and concentrated to 1 or 2 ml. Acidic oligosaccharides of these different fractions were submitted to preparative chromatography

on Whatman No. 3 paper using the solvents described above. After localization of oligosaccharides by staining lateral bands, the products were eluted with water and the solutions were lyophilized.

Fractionation of acidic oligosaccharides and glycopeptides from pathological urines

With pathological urines, which generally contain large amounts of sugars, a simplified procedure, outlined in Fig. 1, was used.

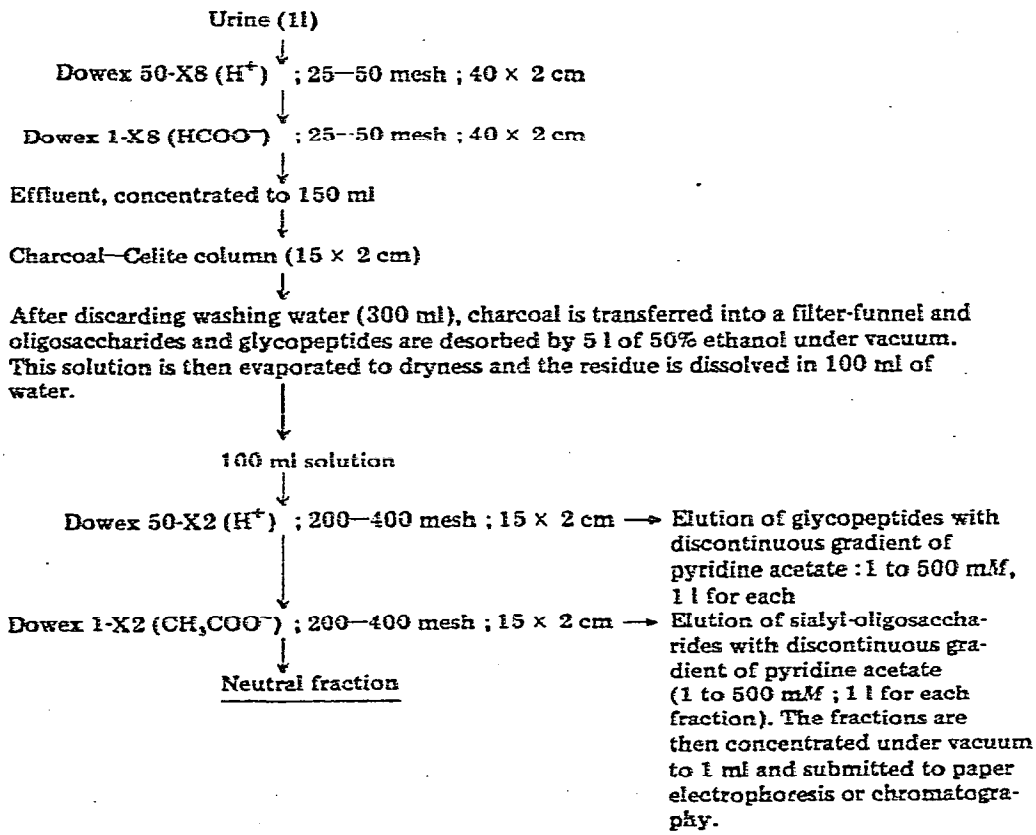

Fig. 1. Scheme for the fractionation of urinary sialyl-oligosaccharides and glycopeptides.

RESULTS

Fractionation of acidic oligosaccharides from normal urine.

Fig. 2 reveals the complex composition of urinary oligosaccharides. Only ethanolic fractions eluted after a 7.5% concentration contain acidic oligosaccharides, which were characterized by paper electrophoresis in pyridine acetate buffer (pH 5.4). The fractionation of these oligosaccharides on an anion ex-

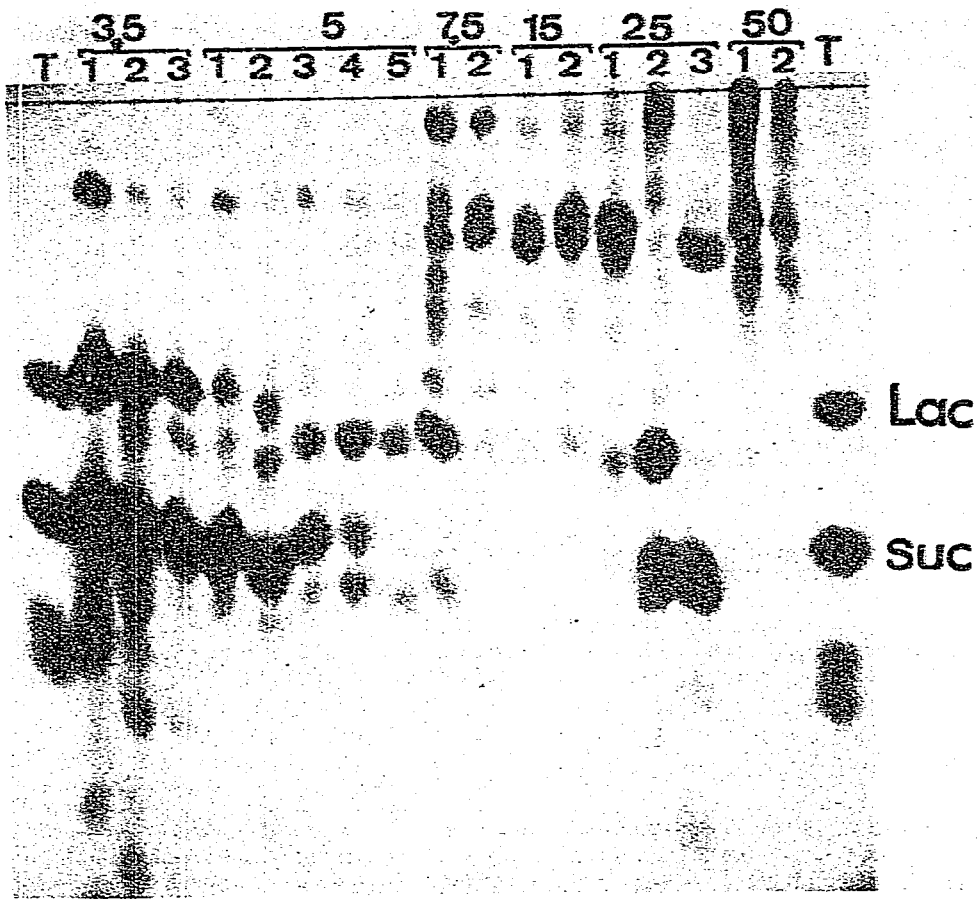

Fig. 2. Normal urine: paper chromatography of oligosaccharides desorbed from a charcoal—Celite column with discontinuous gradient of ethanol (3.5—50%). Solvent S1, development for 5 days. Standard(T): lactose (Lac) and sucrose (Suc).

changer leads to the isolation of a large number of components, as is shown in Figs. 3 and 4. The volume of each sample submitted to paper chromatography varies from 0.5 to 2 ml and corresponds to a 10,000 to 40,000-fold concentrated urine. Finally, preparative paper chromatography using solvent S2 for 2—20 days furnished 45 components with satisfactory purity, as can be seen in Figs. 5 and 6. These different fractionation steps are described in Figs. 2—6.

Oligosaccharides 1—16 were isolated from 7.5, 15 and 25% ethanol fractions. Compounds 17—45 were obtained from the 50% ethanol fraction which was submitted, in a second step, to anion-exchanger separation using the following pyridine acetate concentrations: compounds 17 and 18, 2 m*M*; compounds 19—26, 5 m*M*; compounds 27—31, 10 m*M*; compounds 32—36, 20 m*M*; compounds 37—44, 50 m*M*; and compound 45, 100 m*M*.

Oligosaccharides 2—16 are di- and trisaccharides, as shown in Table I and

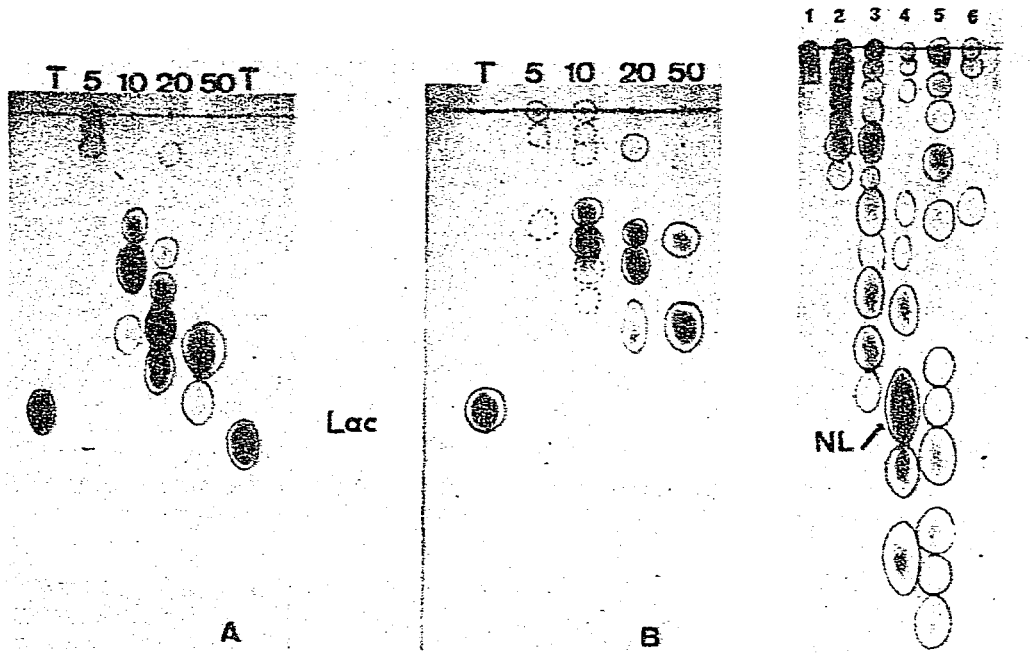

Fig. 3. Normal urine: paper chromatography of acidic oligosaccharides eluted from charcoal with 7.5% ethanol (A) and 15% ethanol (B) and fractionated on an anion exchanger. Pyridine acetate concentrations: 5, 10, 20 and 50 m*M*. Solvent S2, development for 20 h. Standard (T): lactose (Lac).

Fig. 4. Normal urine: paper chromatography of acidic oligosaccharides eluted from charcoal with 50% ethanol and fractionated on an anion exchanger. Pyridine acetate concentrations: 1, 2 m*M*; 2, 5 m*M*; 3, 10 m*M*; 4, 20 m*M*; 5, 50 m*M*; 6, 100 m*M*. Solvent S2, development for 5 days. Samples were concentrated 20,000-fold compared with the volume of treated urine. NL = 3'-neuraminyllactose.

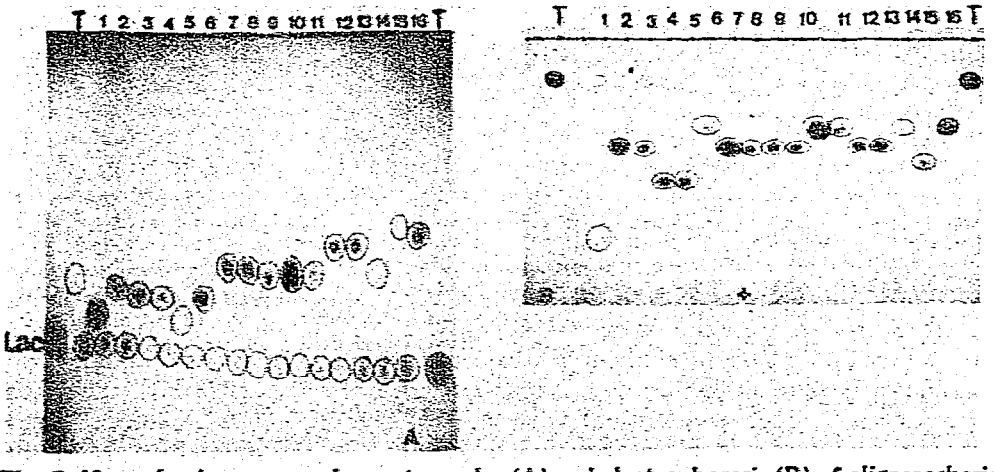

Fig. 5. Normal urine: paper chromatography (A) and electrophoresis (B) of oligosaccharides 1—16 isolated from 20 l of urine. A, Solvent S1, development for 20 h; B, buffer of pH 5.4. Standard(T) = lactose (Lac).

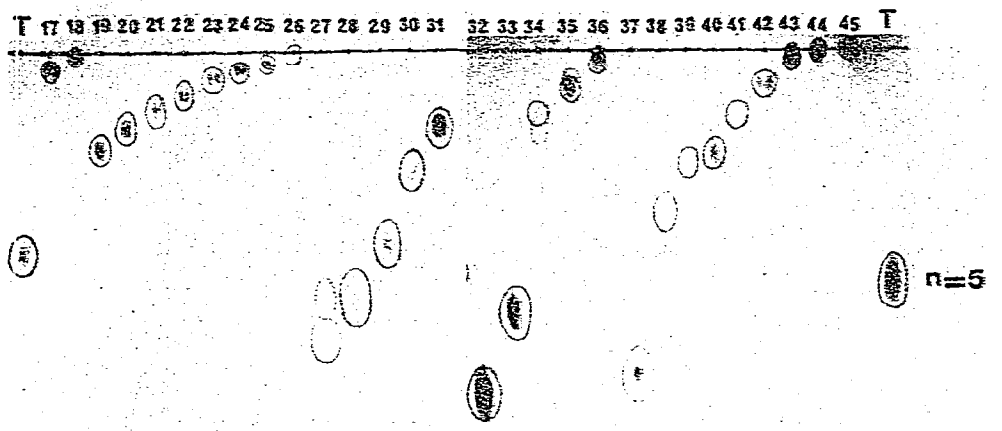

Fig. 6. Normal urine: paper chromatography of oligosaccharides 17—45 isolated from 20 l of urine. Solvent S2, development for 6 days. Lateral standard (T) is a pentasaccharide.

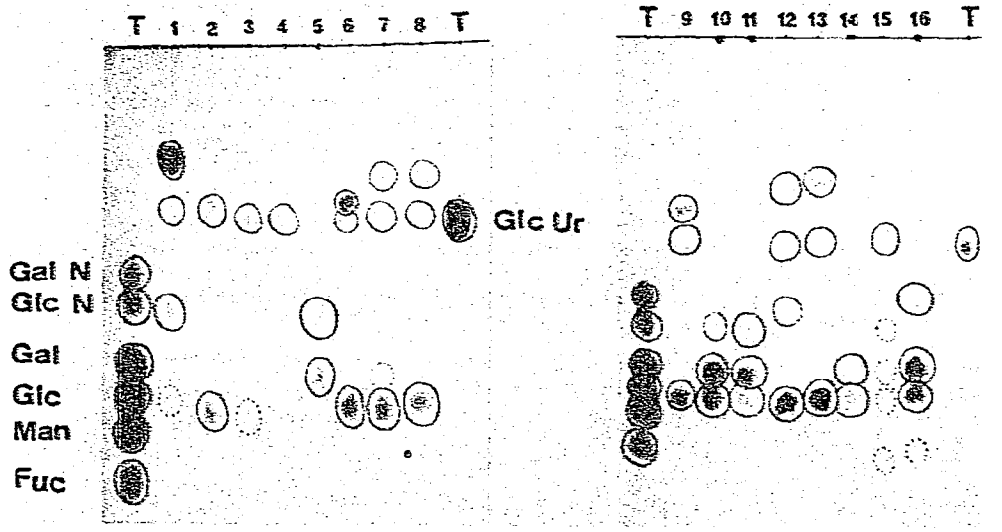

Fig. 7. Normal urine: paper chromatography in solvent S2 of trifluoroacetic acid hydrolysates of oligosaccharides 1—16. Standard (T): galactosamine (GalN); glucosamine (GlcN); galactose (Gal); glucose (Glc); mannose (Man); fucose (Fuc); glucuronic acid (GlcUr).

Fig. 7. Only compounds 1, 2, 6, 7, 8, 9 and 13 were obtained absolutely pure. Their structures are now under investigation.

Compounds 17—45 can be divided into three groups (Fig. 8 and Table I):

(i) neuramic acid and glucose-containing oligosaccharides (27—33), the sugar composition of which is identical with that of glycolipids; (ii) neuramic acid and mannose-containing oligosaccharides (17—26, 34—36 and 43—45), which are related to the glycoprotein catabolism and all of which possess an N-acetyl-

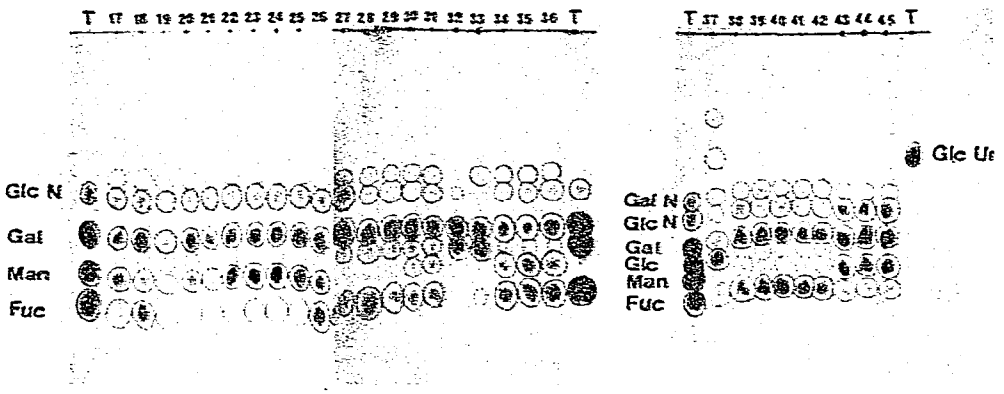

Fig. 8. Normal urine: paper chromatography of trifluoroacetic acid hydrolysates of oligosaccharides 17—45. Solvent S2. Standards: see Fig. 7.

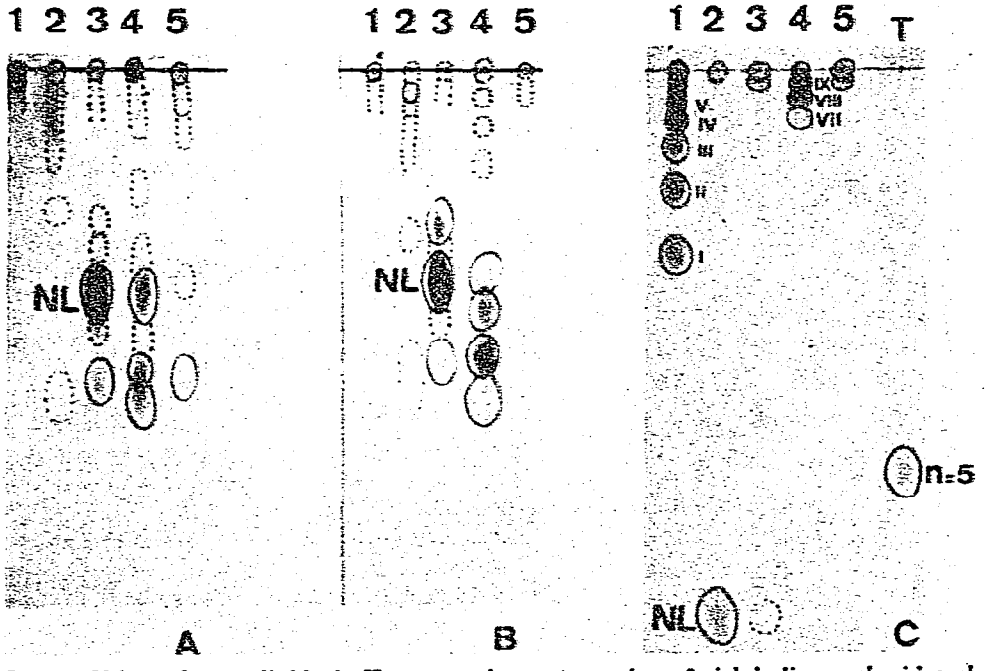

Fig. 9. Urine of mucolipidosis II: paper chromatography of sialyl-oligosaccharides eluted from an anion exchanger with a discontinuous gradient of pyridine acetate: 1, 5 m*M*; 2, 10 m*M*; 3, 20 m*M*; 4, 50 m*M*; 5, 100 m*M*. *A and B, normal urines; C, mucolipidosis II.* Solvent S2, development for 48 h (A and B) and 4 days (C). Samples of normal urine were concentrated 10-fold compared with samples of mucolipidosis II. NL = 3-neuraminyllactose.

glucosamine residue at the reducing end; and (iii) neuramic acid-free glycopeptides (38—42). These last compounds are characterized by the absence of mannose and by the presence of N-acetylgalactosamine, N-acetylglucosamine and galactose. Hence they are products of the catabolism of "mucine-like" constituents.

TABLE I

COMPOSITION OF ACIDIC OLIGOSACCHARIDES AND GLYCOPEPTIDES ISOLATED FROM NORMAL URINE

Gal = galactose; Glc = glucose; Man = mannose; Fuc = fucose; GalNAc = N-acetylgalactosamine; GlcNAc = N-acetylglucosamine; NANA = N-acetylneuraminic acid; GlcUr = glucoronic acid.

Compound	Amount isolated from 20 l of urine (mg)	Molar ratio												Sugar in reducing position
		Gal	Glc	Man	Fuc	GalNAc	GlcNAc	NANA	GlcUr	Asp	Ser	Thr	Gly	
1	35						0.91		1					GlcNAc
2	10		1.12						1					Glc
3	34								+					
4	26								+					
5	57	1					1.05	0.90						GlcNAc
6	53		1.16						1					Glc
7	20		1.05						1					Glc
8	9		1.15						1					Glc
9	10		1.18						1					Glc
10	205	1	0.85				0.20	0.90						Glc + GlcNAc
11	9	1	0.70				0.35	0.95						Glc + GlcNAc
12	9		1.02				0.2		1					Glc + GlcNAc
13	5		1.05						1					Glc
14	4.2	1	1.12					0.85						Glc
15	15								+					
16	41	1	1.06			0.2		0.90						Glc
17	3	1.5		2.85	0.80	0.1	4.08	1						GlcNAc
18	9.5	7.14		2.96	5.60	2.2	8.5	1						GlcNAc
19	2	2	0.4	0.94	0.60		2.1	1						GlcNAc
20	3.5	1.5	0.3	1.12	0.3		1.85	1						GlcNAc
21	2.1	1.80	0.2	1.75	0.09		2.10	1						GlcNAc
22	4.3	1.60	0.2	2.2	0.3		2.50	1						GlcNAc
23	5.2	1.95	0.3	2.85	0.4		3.01	1						GlcNAc
24	3.5	2.1		2.85	0.60		3.25	1						GlcNAc
25	5	2.70		3.30	0.85		3.85	1						GlcNAc

26	5.6	2		1.46	2.2	1	2.4	0.1		0.3	0.5	0.3	0.1	ND
27	3													
28	4	1	0.08		0.1	0.1	0.1	1						GlcNAc
29	4	1.25	0.45		0.25	0.1	1.05	1						GlcNAc
30	4.5	1.70	0.5	0.3	0.4	0.6	0.9	1						ND
31	4.6	2.50	0.95	1	0.85	0.8	1.30	1						GlcNAc
32	60	1	0.9				0.06	1						Glc
33	6.5	1.1	0.90		0.05	0.15		1						Glc
34	3.4	1		0.8	1.1	0.6	1.70			1				
35	2	1.12		2.1	1.9	0.6	1.90			1				
36	6.2	1.9		1	1.9	0.80	2.10			1				
37	1.5													
38	3.5	1	0.1	0.1	1	0.6	0.7			0.7	0.7	0.1		
39	2	1	0.12	0.1	1.50	0.90	0.60			0.65	0.6	0.5	0.4	
40	4	1.1	0.09	0.09	2	0.4	1.05			0.85	0.42	0.7	0.2	
41	2.4	1	0.1	0.1	2	0.8	0.9			1.05	0.9	0.3	0.1	
42	4	2	0.15	0.10	2	1.08	1.63			1.11	0.8	0.7	0.4	
43	5.5	3.15		3.15	1.25	0.3	5.1	2						GlcNAc
44	13	2.90		2.91	0.95	0.2	4.90	2						GlcNAc
45	12	3		2.90	0.90	0.09	5.05	1.85						GlcNAc

Pathological urines

The application of the procedure outlined in Fig. 1 led us to characterize sialyl-oligosaccharides or glycopeptides accumulated in the urine of patients with inborn metabolic diseases.

(i) Mucolipidosis II, or "I-cell disease" [8]. We studied the urine of seven patients with I-cell disease and, in all instances, we found an important excretion of sialyl-oligosaccharides (Figs. 9 and 10 and Table II), the level of which was 30—100-fold the normal values. The structures of nine of these compounds were determined [9, 10] and are shown in Fig. 11.

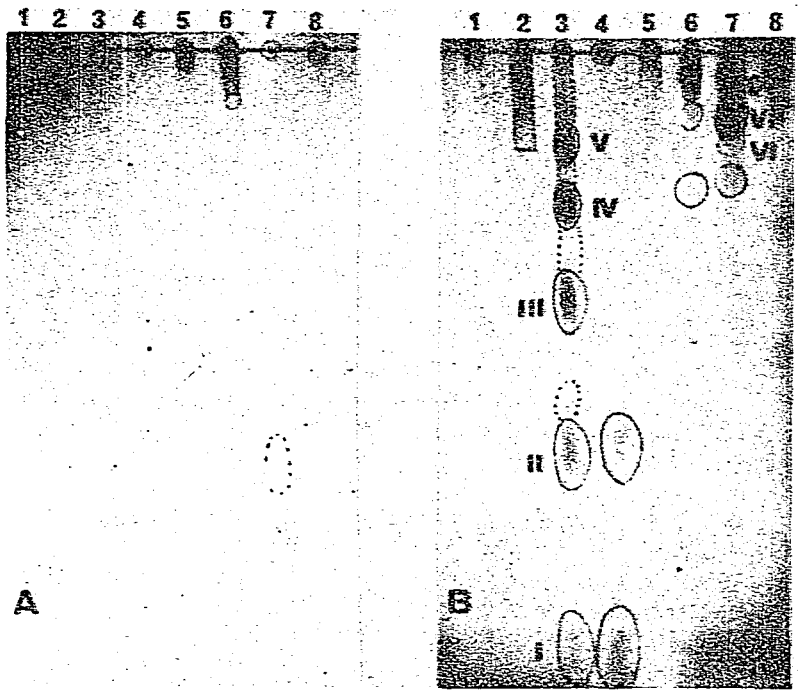

Fig. 10. Urine of mucolipidosis II: paper chromatography of acidic oligosaccharides in a second case of mucolipidosis II. Solvent S2, development for 14 days. A, normal urine; B, mucolipidosis II. Pyridine acetate concentrations: 1, 1 m*M*; 2, 2 m*M*; 3, 5 m*M*; 4, 10 m*M*; 5, 20 m*M*; 6, 50 m*M*; 7, 100 m*M*; 8, 200 m*M*.

(ii) New types of mucolipidosis. Mucolipidosis W. (Mande, unpublished observation) was observed in two children (Laura and Pierre Alexandre W.) and is very similar to I-cell disease, except the fact that the activities of many hydrolases increase in the cells but are normal in the serum. Neuraminidase activity in leucocytes was 2—4% of the normal level.

Mucolipidosis De P. (Durand, unpublished observation) is characterized only by a red spot in the bottom of the eyes and by a dyschromatopsy. The patients (22 and 9 years' old) do not exhibit other significant clinical symptoms or mental retardation. Neuraminidase activities in leucocytes were 18 and 22% of the normal levels in these patients.

TABLE II

SUGAR COMPOSITION OF OLIGOSACCHARIDES ISOLATED FROM URINE OF A PATIENT WITH MUCOLIPIDOSIS II

Abbreviations as in Table I.

Oligosaccharides*	Amount (mg)**	Molar ratio***				Monosaccharide in reducing position
		Gal	Man	GlcNAc	NANA	
I	8	0.91	2	2.07	1.05	GlcNAc
II	11	1.04	2	1.78	1.02	GlcNAc
III	14	0.95	3	2.28	0.95	GlcNAc
IV	15	1.69	3	2.90	0.95	GlcNAc
V	12	1.89	3	3.09	0.96	GlcNAc
VI	5	1.72	2	2.95	1.96	GlcNAc
VII	7	1.75	3	2.92	1.89	GlcNAc
VIII	16	1.91	3	2.89	1.91	GlcNAc
IX	23	2.12	3	3.08	1.96	GlcNAc

* See Fig. 10.
** Milligrams of oligosaccharide isolated per litre of urine by paper chromatography. The amount of isolated product is approximately 60% of urinary material. The level of these oligosaccharides in normal urine is less than 0.5 mg/l.
*** On the basis of 2 or 3 mannose residues.

These two new types of mucolipidosis are characterized by an important excretion of sialyl-oligosaccharides, 300- to 500-fold the normal value (Fig. 12 and Table III), the structures of which are identical with those of I-cell disease (Fig. 11).

(iii) Fucosidosis [11]. With fucosidosis, accumulated material was found mainly in "glycopeptidic fractions" eluted from the cation exchanger and in the "neutral fraction". Electrophoresis of "glycopeptidic fractions" showed abnormal constituents eluted by 1.2 and 5 m*M* pyridine acetate (Fig. 13). Paper chromatography of these fractions furnished five glycopeptides in a pure state (Fig. 14 and Table IV). The neutral fraction (Fig. 15) contained a glycopeptide (GP-6), which remained at the starting point and was purified by paper chromatography in 40 days. The structures of these components have been previously described [12] and are shown in Fig. 16.

DISCUSSION

The fractionation procedures described allow us to characterize, in urine, carbohydrate compounds resulting from the catabolism of glycoconjugates. In normal urine, the level of each component (less than 0.2 mg/l) was too low for structural studies to be undertaken.

These procedures were applied in studies of mucolipidosis and fucosidosis and can be used as a method of diagnosis. Mucolipidosis II, W. and De P. are characterized by a partial or total lack of neuraminidase activity [9, 13] and

I α-NANA-(2 → 3)-β-Gal-(1 → 4)-β-GlcNAc-(1 → 2)-α-Man-(1 → 3)-β-Man-(1 → 4)-GlcNAc

II α-NANA-(2 → 6)-β-Gal-(1 → 4)-β-GlcNAc-(1 → 2)-α-Man-(1 → 3)-β-Man-(1 → 4)-GlcNAc

III α-NANA-(2 → 6)-β-Gal-(1 → 4)-β-GlcNAc-(1 → 2)-α-Man-(1 → 3)
β-Man-(1 → 4)-GlcNAc
α-Man-(1 → 6)

IV α-NANA-(2 → 3)-β-Gal-(1 → 4)-β-GlcNAc-(1 → 2)-α-Man-(1 → 3)
β-Man-(1 → 4)-GlcNAc
β-Gal-(1 → 4)-β-GlcNAc-(1 → 2)-α-Man-(1 → 6)

V α-NANA-(2 → 6)-β-Gal-(1 → 4)-β-GlcNAc-(1 → 2)-α-Man-(1 → 3)
β-Man-(1 → 4)-GlcNAc
β-Gal-(1 → 4)-β-GlcNAc-(1 → 2)-α-Man-(1 → 6)

VI α-NANA-(2 → 6)-β-Gal-(1 → 4)-β-GlcNAc-(1 → 2)
α-Man-(1 → 3)-β-Man-(1 → 4)-GlcNAc
α-NANA-(2 → 3)-β-Gal-(1 → 4)-β-GlcNAc-(1 → 4)

VII α-NANA-(2 → 3)-β-Gal-(1 → 4)-β-GlcNAc-(1 → 2)-α-Man-(1 → 3)
β-Man-(1 → 4)-GlcNAc
α-NANA-(2 → 3)-β-Gal-(1 → 4)-β-GlcNAc-(1 → 2)-α-Man-(1 → 6)

VIII α-NANA-(2 → 6)-β-Gal-(1 → 4)-β-GlcNAc-(1 → 2)-α-Man-(1 → 3)
β-Man-(1 → 4)-GlcNAc
α-NANA-(2 → 3)-β-Gal-(1 → 4)-β-GlcNAc-(1 → 2)-α-Man-(1 → 6)

IX α-NANA-(2 → 6)-β-Gal-(1 → 4)-β-GlcNAc-(1 → 2)-α-Man-(1 → 3)
β-Man-(1 → 4)-GlcNAc
α-NANA-(2 → 6)-β-Gal-(2 → 4)-β-GlcNAc-(1 → 2)-α-Man-(1 → 6)

Fig. 11. Structure of the nine major oligosaccharides isolated from the urine of mucolipidosis II, W and De P. [9, 10].

by a correlative accumulation of sialyl-oligosaccharides in urine. The structures of nine of them have been elucidated, but more than 20 different components remain to be identified. If we assume that these oligosaccharides originate from all of the glycoproteins of the organism, we should be able to predict all of the possible structures of glycans.

It is also interesting to note that all of these oligosaccharides possess an N-acetylglucosamine residue in the reducing position. This result is in good agreement with the hypothesis of the existence of a β-endo-N-acetylglucosaminidase which is able to split glycans even if they are sialylated [14, 15], as with glycans of the "oligomannosidic type" [14, 16]. This enzyme remains to be characterized among mammalians.

TABLE III

SUGAR COMPOSITION OF OLIGOSACCHARIDES ISOLATED FROM URINE OF A PATIENT WITH A NEW TYPE OF MUCOLIPIDOSIS (LAURA W.)

Abbreviations as in Table I.

Oligosaccharides*	Amount* (mg/l)	Molar ratio			
		Gal	Man	GlcNAc	NANA
I	28	1.02	2	2.12	0.94
II	125	1.01	2	1.89	0.96
III	15	1.06	3	1.87	1.11
IV	5	2.24	3	3.20	0.96
V	10	2.20	3	3.00	1.10
VI	15	1.72	2	2.95	2.18
VII	24	1.74	3	2.76	1.95
VIII	48	1.72	3	2.84	2.20
IX	160	2.12	3	2.99	2.08
X	17	2.84	3	3.66	2.66
XI	8	3.20	3	3.75	2.90

* See Fig. 12.

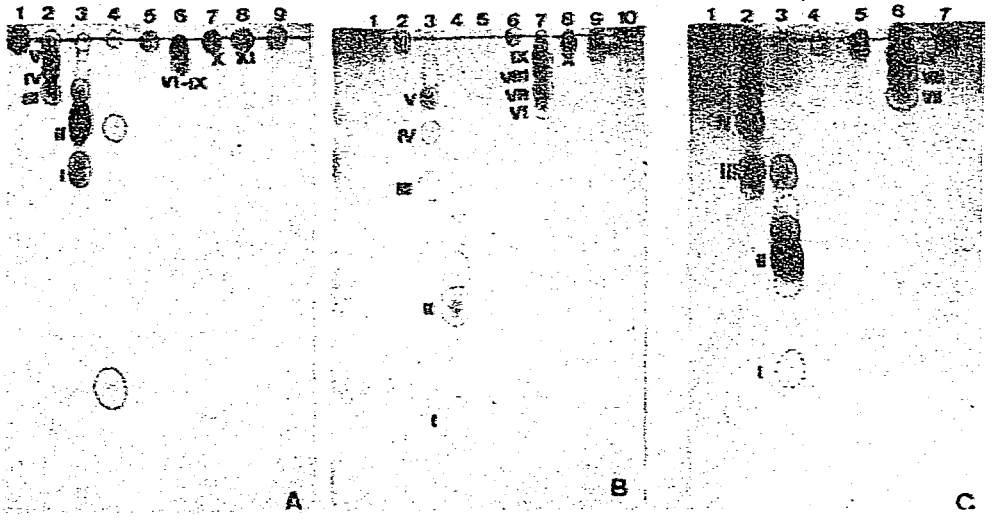

Fig. 12. Paper chromatography of acidic oligosaccharides characterized in urine of patients with two new types of mucolipidosis. A and B, mucolipidosis W (Laura and Pierre Alexandre W.); C, mucolipidosis De P. Solvent S2, development for 6 days (A) or 14 days (B and C). Samples were concentrated 300-fold compared with the initial volume of urine. Pyridine acetate concentrations, see Fig. 10.

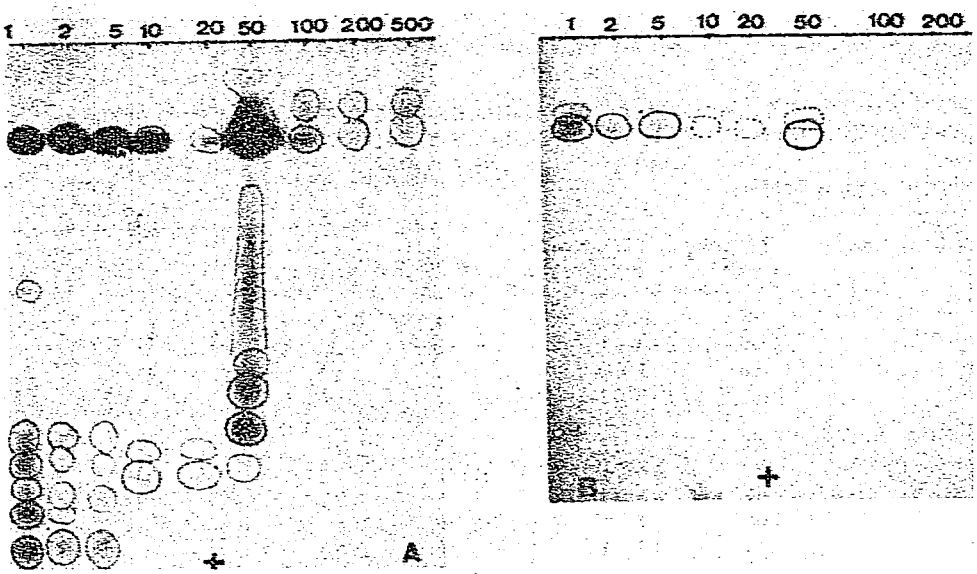

Fig. 13. Urine of fucosidosis: paper electrophoresis of "glycopeptidic fractions" eluted from a cation exchanger with a discontinuous gradient of pyridine acetate (1—500 m*M*). A, Stained with ninhydrin; B, stained with aniline oxalate.

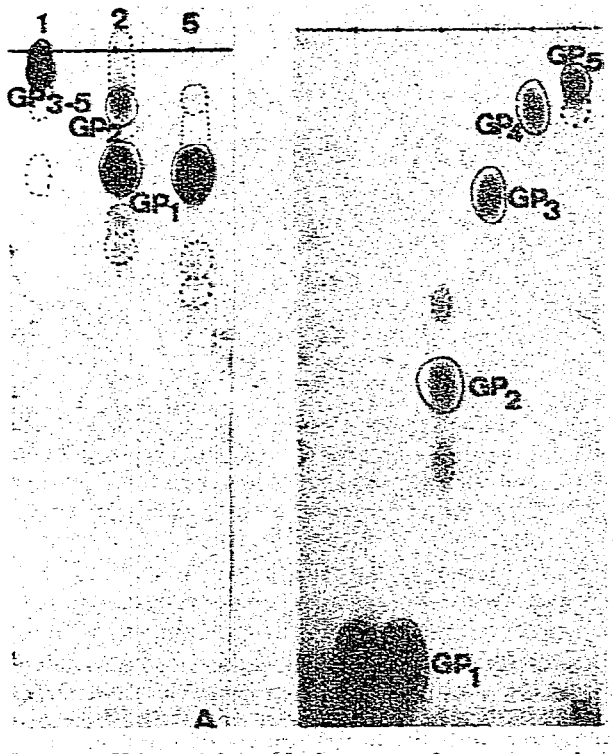

Fig. 14. Urine of fucosidosis: paper chromatography of glycopeptides. A, Fractions eluted from a cation exchanger with 1, 2 and 5 m*M* pyridine acetate; B, glycopeptides isolated by paper chromatography. Solvent S2, development for 20 h (A) or 4 days (B).

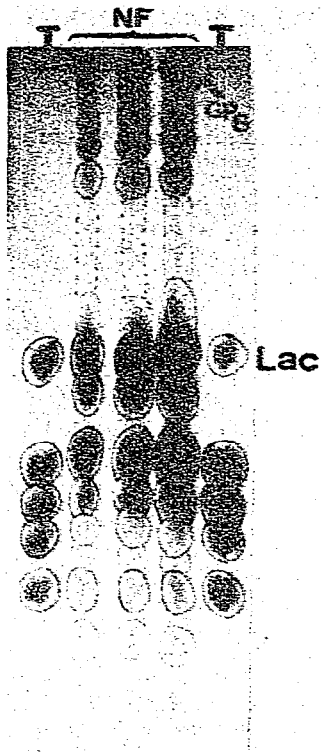

Fig. 15. Urine of fucosidosis: paper chromatography of "neutral fraction" (NF). Solvent S2, development for 18 h. The arrow indicates glycopeptide GP-6. T = standard. Lac = lactose.

TABLE IV

SUGAR COMPOSITION OF GLYCOPEPTIDES ISOLATED FROM URINE OF A PATIENT WITH FUCOSIDOSIS

Abbreviations as in Table I.

Glycopeptide	Amount* (mg/l)	Molar ratio**				
		Gal	Glc	Man	Fuc	GlcNAc
GP-1	96	—	—	—	1.05	0.96
GP-2	7	0.88	0.07	—	0.96	0.90
GP-3	22	—	—	1.88	0.99	1.82
GP-4	10	0.95	—	1.94	1.86	2.84
GP-5	7	1.14	—	1.92	1.96	2.90
GP-6	98	3.90	—	3.05	5.02	6.05

* See Table II.
** On the basis of 1 aspartic acid residue.

GP-1 α-Fuc-(1 → 6)-β-GlcNAc → Asn

GP-3 α-Man-(1 → 6)-β-Man-(1 → 4)-β-GlcNAc-(1 → 4)
β-GlcNAc → Asn
α-Fuc-(1 → 6)

GP-4 α-Fuc-(1 → 3)-β-Gal-(1 → 4)-β-GlcNAc-(1 → 2)-α-Man-(1 → 6)-β-Man-(1 → 4)-β-GlcNAc-(1 → 4)
β-GlcNAc → Asn
α-Fuc-(1 → 6)

GP-5a α-Fuc-(1 → 6)-β-Gal-(1 → 4)-β-GlcNAc-(1 → 2)-α-Man-(1 → 6)-β-Man-(1 → 4)-β-GlcNAc-(1 → 4)
β-GlcNAc → Asn
α-Fuc-(1 → 6)

GP-5b α-Fuc-(1 → 6)-β-Gal-(1 → 4)-β-GlcNAc-(1 → 2)-α-Man-(1 → 6)-β-Man-(1 → 4)-β-GlcNAc-(1 → 4)
β-GlcNAc → Asn
α-Fuc-(1 → 3)

Fig. 16. Structure of glycopeptides isolated from the urine of fucosidosis [12].

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REFERENCES

1 G. Strecker, B. Bayard and J. Montreuil, C.R. 6e Symp. Intern. Chromatogr. Electrophor., Bruxelles 1970, Presses Acad. Eur., Bruxelles, 1971, p. 324.
2 G. Strecker, C. Trentesaux-Chauvet, A. Poitau and J. Montreuil, Biochimie, 58 (1976) 805.
3 G. Strecker and J. Montreuil, Clin. Chim. Acta, 33 (1971) 395.
4 G. Strecker, B. Fournet, S. Bouquelet, J. Montreuil, J.L. Dhondt and J.P. Farriaux, Biochimie, 58 (1976) 579.
5 J. Montreuil and G. Spik, Microdosages des glucides, Monographies Lab. Chim. Biol. Fac. Sci. Lille I, Fase. 1, 1968.
6 J.P. Zanetta, W.C. Breckenridge and G. Vincendon, J. Chromatogr., 69 (1972) 291.
7 R.L. Whistler and D.F. Durso, J. Amer. Chem. Soc., 44 (1950) 166.
8 J.G. Leroy, R.I. De Mars and J.M. Opitz, Birth Defects, Orig. Artic. Ser., 5 (1969) 174.
9 G. Strecker, B. Fournet, T. Hondi-Assah, G. Spik, J. Montreuil, P. Maroteaux, P. Durand and J.P. Farriaux, Biochim. Biophys. Acta, 444 (1976) 349.
10 G. Strecker, M.C. Peers, J.C. Michalski, B. Fournet, G. Spik, J. Montreuil, J.P. Farriaux, P. Maroteaux and P. Durand, Eur. J. Biochem., 75 (1977) 391.
11 P. Durand, C. Borrone and G. Della Cella, J. Pediatr., 75 (1969) 665.
12 G. Strecker, B. Fournet, G. Spik, J. Montreuil, P. Durand et M. Tondeur, C.R. Acad. Sci., Ser. D, 284 (1977) 85.
13 G. Strecker, J.C. Michalski, J.P. Farriaux and J. Montreuil, Biomédecine, 25 (1976) 238. 238.
14 J. Montreuil, Pure Appl. Chem., 42 (1975) 433.
15 G. Strecker et J. Montreuil, C.R. Acad. Sci., in press.
16 T. Tai, K. Yamashita, M. Ogata-Arakawa, N. Koide, T. Muramatsu, S. Iwashita, Y. Imoue and A. Kobata, J. Biol. Chem., 250 (1975) 8569.