[Chem. Pharm. Bull. 20(5)1070—1078(1972)]

UDC 547.458.2.08 : 543.544

Gas Chromatographic Analysis of Disaccharides. II.[1] Analysis of Disaccharides in Human Body Fluids[2]

Hiroshi Nakamura and Zenzo Tamura

Faculty of Pharmaceutical Sciences, University of Tokyo[3]

(Received November 16, 1971)

Analysis of disaccharides in the field of clinical chemistry has been done exclusively by paper chromatography. Recently, enzymatic analysis of lactose in the blood and urine was performed by a galactose-oxidase method after hydrolysis of lactose by β-galactosidase.[4] Another recent progress in this field is the use of a carbohydrate autoanalyzer.[5] However, these anlytical methods are lack of sensitivity and selectivity to deal with biological materials. Huttunen and Miettinen[6] analyzed oligosaccharides containing neuraminic acid in human urine by gas chromatography, while gas chromatographic analysis of neutral disaccharides in body fluids has never been reported. In the previous paper,[1] we established the gas chromatographic analysis of disaccharides as trimethylsilyl (TMS) and trifluoroacethyl (TFA) derivatives of the corresponding disaccharide alditols. We extended the study further in this work to the analyses of disaccharides in human body fluids.

Experimental

1. Reagents——Urease (Jack Bean Meal) was purchased from Daiichi Pure Chemical Co., Ltd., Tokyo. Sodium borohydride ($NaBH_4$, GR) was a product of E. Merck. Sodium fluoride (NaF, GR) was from Nakarai Chemicals, Ltd., Kyoto. 3-O-Methyl-α-D-glucose (mp 163—165°) was synthesized after the method of Glen, *et al.*[7] Other carbohydrates used in this study were all guaranteed grade of commercial sources. Ethanol (EtOH) and methanol (MeOH) were distilled once before use. Reagents and solvents for trimethylsilylation and trifluoroacethylation were same and were treated similarly as in the previous paper.[1]

2. Preparation of Ion Exchanger Column——Amberlite CG-120 (H^+) column: Amberlite CG-120 (type I) was washed with water and placed in a glass tube. The column was washed with 2N NaOH, water, 2N HCl and water. The resin was pipetted into chromatography tube of 8 mm in diameter to form a packed layer of 60 or 120 mm long, with a similar design as reported by Takanashi, *et al.*[8]

Amberlite CG4-B (CH_3COO-) column: Amberlite CG-4B (type I) was washed with water and placed in a glass tube. The column was washed with 2N HCl, water, 2N NaOH and water. Then 10% (v/v) acetic acid was passed through the column and it was washed with water to remove excess acetic acid. The resin was pipetted into tube of 8 mm in diameter up to 60 or 120 mm long.

3. Pretreatment of Sample——Urine: The subjects were 14 healthy male adults. They had usual dinner and were fasted for 12 hr and made their bladder empty. The urine accummulated within 2 hr period thereafter was used for analysis.

The established procedure was as follows. One ml of urine was heated for 5 min in a boiling water bath and after cooling with tap water was added with 0.5 ml of the internal standard solution containing cellobiose or α,α-trehalose (10—500 μg). Cellobiose was used for the analysis of lactose and maltose, and α,α-trehalose was for that of sucrose. The sample was incubated with 5 mg of urease for 30 min at 45°. After cooling to the room temperature, the urease digest obtained was passed through columns of Amberlite CG-120 (H^+,

1) Part I: H. Nakamura and Z. Tamura, *Chem. Pharm. Bull.* (Tokyo), **18**, 2314 (1970).
2) This work was presented at the 91th Annual Meeting of Pharmaceutical Society of Japan, Fukuoka, April 1971.
3) Location: *Hongo-7-3-1, Bunkyo-ku, Tokyo.*
4) B. Tengström and L. Wranne, *Scand. J. Clin. Lab. Invest.*, **22**, 137 (1968).
5) R.L. Jolley and M.L. Freeman, *Clin. Chem.*, **14**, 538 (1968).
6) J.K. Huttunen and T.A. Miettinen, *Anal. Biochem.*, **29**, 441 (1969).
7) W.L. Glen, G.S. Myers and G.A. Grant, *J. Chem. Soc.*, (1951) 2568.
8) S. Takanashi, I. Matsunaga and Z. Tamura, *J. Vitaminol.*, **16**, 132 (1970).

0.8×12 cm) and Amberlite CG-4B (CH_3COO^-, 0.8×12 cm), eluting with 20 ml of water. The effluent was evaporated to dryness.[9] In the case of the analysis of sucrose, the residue obtained was directly used for gas chromatography. In the case of the analysis of lactose and maltose, the residue was added with 0.5 ml of water and 0.5 ml of 1% $NaBH_4$ and the reduction of the carbohydrates was performed for 30 min at room temperature. The excess reagent was destoroyed by adding with 0.5 ml of Amberlite CG-120 (H^+) and the reaction mixture was filtered on a glass filter to remove the resin. After washing the resin with 0.5 ml

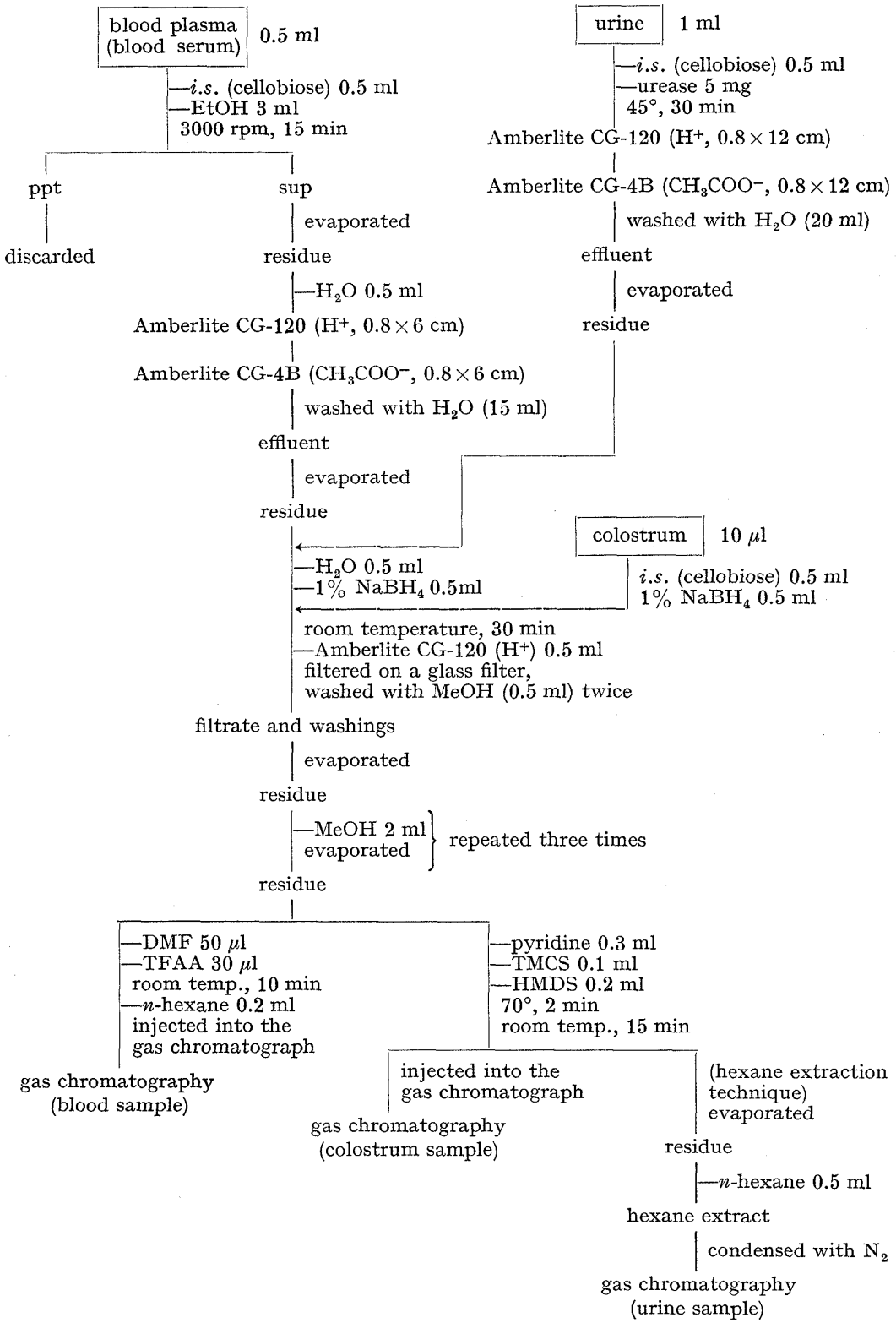

Chart 1. Gas Chromatographic Analysis of Disaccharides in Human Body Fluids

of MeOH twice, the combined filtrate and the washings were evaporated to dryness. To the residue, 2 ml of MeOH was added and was evaporated to dryness to remove borate as trimethylborate. The final treatment was repeated three times.

Blood: The subjects were 14 normal individuals, 13 adult diabetics, 13 lying-in women and 8 patients afflicted with various diseases. They took their customary dinner and were fasted for 12 hr by omitting the breakfast on the next day. The blood was drawn from arm vein. Blood plasma was prepared by adding NaF to the blood in a ratio of 10 mg/ml and by centrifuging for 10 min at 3000 rpm with a laboratory centrifuge to remove corpuscles. Preparation of blood serum was made by a conventional method.

The standard procedure for plasma and serum was as follows. To 0.5 ml of the blood specimen in a centrifuge tube or a test tube, 0.5 ml of the aqueous solution containing 3-*O*-methyl-α-D-glucose (500 μg) and cellobiose (0.2—10 μg) was added as internal standards for mono-, and disaccharides. The resulted solution was deproteinized by adding with 3 ml of EtOH and by centrifuging for 15 min at 3000 rpm. The supernatant was evaporated to dryness in 20 ml round flask, added with 0.5 ml of water and deionized by passing through the columns of Amberlite CG-120 (H^+, 0.8×6 cm) and Amberlite CG-4B (CH_3COO^-, 0.8×6 cm). The effluent from 15 ml of water was evaporated to dryness. The successive treatments were same as in the case of urine.

Human Colostrum: The samples were collected at three hospitals from 28 healthy women within one week after delivery and were frozen until they were analyzed. Ten μl of colostrum specimen was added with 500 μg of cellobiose in 0.5 ml of water as an internal standard, added with 0.5 ml of 1% $NaBH_4$ and was stand for 30 min at room temperature. The successive treatments were same as in the case of urine.

The overall procedures for gas chromatographic analysis of disaccharides in clinical materials are summarized in Chart 1.

4. Gas Chromatography——Trimethylsilylation of the samples prepared from urine and colostrum was carried out with trimethylchlorosilane (TMCS, 0.1 ml) and hexamethyldisilazane (HMDS, 0.2 ml) in anhydrous pyridine (0.3 ml) at 70° for 2 min and then at room temperature for 15 min.[1] An aliquot (*ca.* 2 μl) of the reaction mixture was injected directly into a gas chromatograph. In the analysis of urinary disaccharides, the hexane extraction technique[1] was employed. Gas chromatography was carried out on 2% QF-1 column (1.5×4 mm *i.d.*, on Gas Chrom P) at 160° or on 2% OV-17 column (1.8×4 mm *i.d.*, on Gas Chrom P) at 210° with Shimadzu Gas Chromatograph GC-1B or GC-4APF equipped with a hydrogen flame ionization detector. The flow rates of nitrogen are shown in Figures.

The blood samples were trifluoroacethylated by treating the sample with 50 μl of N,N-dimethylformamide (DMF) and 30 μl of trifluoroacetic anhydride (TFAA) for 10 min at room temperature.[1] For the purpose of the analysis of the trifluoroacetates of sucrose and disaccharide alditols, the reaction mixture was diluted with 0.2 ml *n*-hexane and 1—2 μl of the diluted solution was used for gas chromatographic sample. Gas chromatography was performed on 0.5% XF-1105 column (2m×3 mm *i.d.*, on Gas Chrom P) at 165° with Hitachi Gas Chromatograph Model 023—5009 equipped with an nickel (^{63}Ni) electron capture detector. The flow rate of nitrogen was 90 ml/min. For identification of disaccharides in blood, other two stationary phases, 2% OV-1 and 2% QF-1, were also used with the same gas chromatograph under the conditions described in Table IV.

The trifluoroacetates of monosaccharide alditols were analyzed on 2% XF-1105 column (1.8×4 mm *i.d.*, on Gas Chrom P) at 125° with Shimadzu Gas Chromatograph GC-4APF. The flow rate of nitrogen was 45 ml/min.

The determinations were performed with the internal standard method by using the peak height ratio of carbohydrate to cellobiose (*i.s.*).

5. Recovery Test from Blood Plasma and Urine——To 0.5 ml of blood plasma or 1.0 ml of urine, known quantities of authentic disaccharides (sucrose, lactose, maltose and melibiose; 2.5—10 μg each for blood plasma, 25—100 μg each for urine) and cellobiose as an internal standard were added. After treatment of the samples according to the procedure shown in Chart 1, the added disaccharides in biological materials were analyzed by two method; the blood disaccharides were analyzed as TFA derivatives on 0.5% XF-1105 column with the electron capture detector and the urinary disaccharides were as TMS derivatives on 2% QF-1 column.

Results

Recovery Test

The results of the recovery tests of disaccharides from blood plasma and urine are shown in Table I. By using the internal standard method, good recovery was achieved in both TFA and TMS derivatives.

9) All the evaporation procedure was carried out throughout this investigation with a rotary evaporator *in vacuo*, below 40°.

TABLE I. Receovery of Disaccharides from Human Blood Plasma and Urine

Disaccharide	Added (μg)	Found (μg)					Average	
		1	2	3	4	5	(μg)	Recovery (%)
Sucrose	2.50[a]	2.50	2.58	2.42	2.47	2.74	2.54	101.6
	25.0[b]	25.4	25.7	25.0	24.3	24.1	24.9	99.6
Lactose	2.50[a]	2.90	2.47	2.53	2.41	2.66	2.59	103.6
	25.0[b]	24.1	24.8	24.3	23.7	25.5	24.5	97.9
Maltose	5.00[a]	5.21	4.63	5.19	5.07	4.81	4.98	99.6
	50.0[b]	50.2	50.8	53.1	48.5	52.6	51.0	102.1
Melibiose	10.0[a]	10.4	9.82	10.4	10.1	9.93	10.1	101.3
	100.0[b]	103.5	104.4	107.1	103.1	107.2	105.1	105.1

a) blood plasma (TFA derivatives), *b*) urine (TMS derivatives)

Analysis of Urine

A representative gas chromatogram of normal human urine in the fasting condition is shown in Fig. 1. Sucrose and lactose were regular disaccharides found in urine, however, maltose was scarecely contained in urine. The average content of urinary sucrose was 102 μg/ml (Table II), and that of lactose was 10.1 μg/ml (Table III).

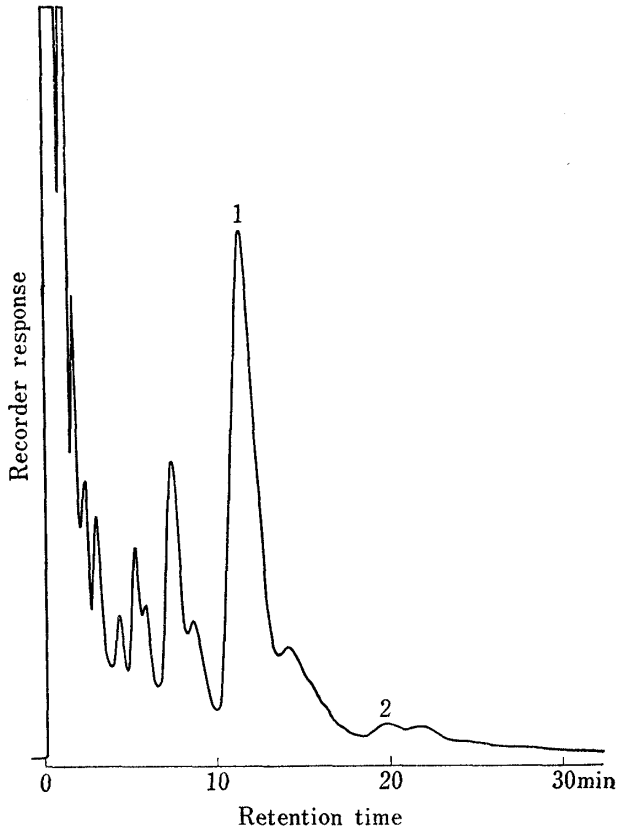

Fig. 1. Representative Gas Chromatogram of Urinary Disaccharides of Healthy Man after 12 hr Starvation (TMS Derivatives)

peak 1: sucrose, 2: lactose
conditions: 2% QF-1 (1.5 m×4 mm *i.d.*), 160°, N_2 45 ml/min

Analysis of Serum and Plasma

Three disaccharides, lactose, sucrose and maltose, were detected in all the blood speciments examined. The tentative identification of them in the blood was performed by comparing the retention times of them with that of authentic disaccharides on three stationary phases, 0.5% XF-1105, 2% QF-1 and 2% OV-1 (Table IV). The concentration of disaccharides in normal persons and diabetics was usually less than 1 μg/ml. Sucrose and maltose in blood were in similar quantities in any groups of normal individuals (Table V), lying-in women (Table VI), diabetics (Table VII) who showed high glucose levels and the patients afflicted with various diseases (Table VIII). However, it is noteworthy that the blood lactose concentration is abnormally high in lying-in women. The representative gas chromatograms obtained from a normal individual and a lying-in woman are shown in Fig. 2a and Fig. 2b, respectively.

Analysis of Human Colostrum

As shown in Fig. 3, lactose is a dominant carbohydrate in colostrum. The lactose contents in human colostrums obtained from 28 healthy women are shwon in Table IX. The

maximal and the minimal lactose concentration were 78.4 mg/ml and 11.1 mg/ml, respectively. No clear difference was found in lactose content of colostrum according to different number of delivery. As shwon in Fig. 4, the lactose content of human colostrum within 24 hr of delivery tends to be smaller than that of colostrum thereafter.

Table II. Excretion of Sucrose in 2 hr-Urine of Healthy Men after 12 hr Starvation

Subject (initial)	Age (yrs.)	Volume (ml)	pH	Sucrose	
				μg/ml	mg
I.M	31	83	5.5	227.0	18.83
H.H	23	100	5.6	184.2	18.42
H.K	28	120	5.7	124.6	14.95
K.I	28	28	5.7	119.3	3.34
K.T	25	192	5.9	112.5	21.60
Y.A	28	103	6.4	99.1	10.20
T.I.	32	N.M.	6.0	93.0	
T.N	24	32	6.9	92.3	2.95
H.N	25	61	5.2	92.2	5.62
H.Y	28	N.M.	N.M.	80.4	
K.M	24	17	5.6	77.2	1.31
K.K	25	98	6.3	61.5	6.03
M.Y	28	180	N.M.	45.9	8.28
H.I	24	450	7.0	24.6	11.07
Average	27	122	6.6	102.4	10.22

N.M.: not measured

Table III. Excretion of Lactose in 2 hr-Urine of Healthy Men after 12 hr Starvation

Subject (initial)	Age (yrs.)	Volume (ml)	pH	Lactose	
				μg/ml	mg
H.N	25	110	N.M.	23.8	2.62
M.Y	28	114	7.6	17.9	2.04
Y.H	25	91	N.M.	14.1	1.28
H.K	28	38	N.M.	7.64	0.29
H.I	24	25	5.7	4.74	0.12
K.M	24	161	N.M.	1.42	0.23
T.N	24	112	6.2	1.13	0.13
Average	25	93		10.1	0.96

N.M.: not measured

Table IV. Retention Times of Disaccharides in Human Blood

	0.5% XF-1105 (2 m, 165°, 90 ml/min)	2% QF-1 (1.5 m, 180°, 55 ml/min)	2% OV-1 (1.5 m, 140°, 60 ml/min)
Blood specimen	5.70 min[a)]	9.78 min[a)]	6.78 min[a)]
	16.4	15.9	8.27
	32.9		
Authentic: Sucrose	5.71	9.78	6.77
Maltitol	16.3	9.79	6.71
Lactitol	33.0	15.9	8.29

a) retention times of TFA derivatives

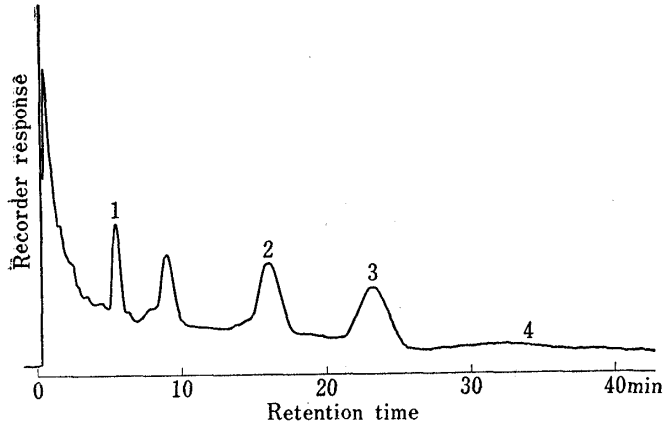

Fig. 2a. Representative Gas Chromatogram of Blood Disacharides of Normal Person after 12 hr Starvation (TFA Derivatives)

peak 1: sucrose, 2: maltose, 3: cellobiose (*i.s.*), 4: lactose
conditions: 0.5% XF-1105 (2m×3 mm *i.d.*), N_2 90 ml/min

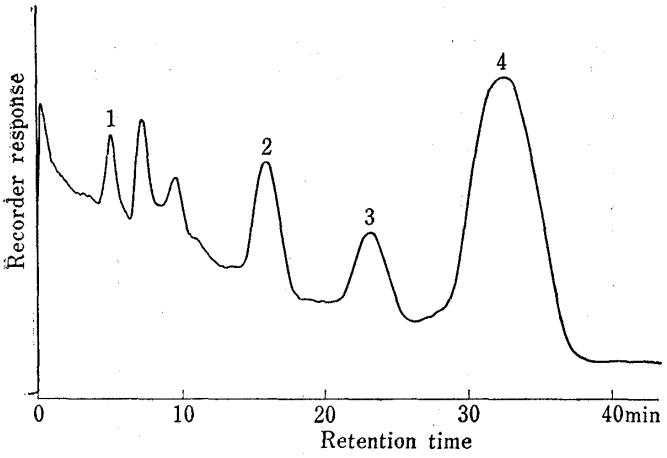

Fig. 2b. Representative Gas Chromatogram of Blood Disaccharides of Lying-in Woman after 12 hr Starvation (TFA Derivatives)

The peaks and the conditions were the same as in Fig. 2a.

Trace amount of fructose was detected in colostrum as well as glucose, inositol and lactose (Fig. 3). Occurrence of fructose in colostrum was indicated by the presence of TMS-mannitol after reduction of the sample and confirmed by the presence of the peak corresponding to authentic fructose and the absence of the peak corresponding to authentic mannitol on gas chromatogram of TFA derivatives of colostrum specimen without reduction.

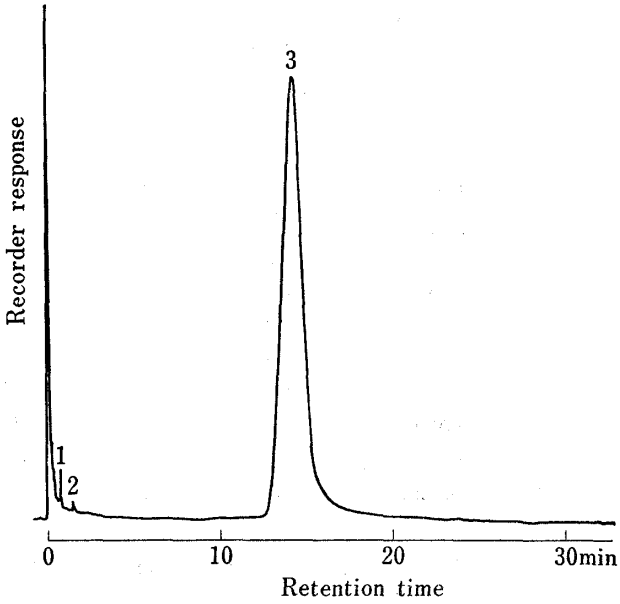

Fig. 3. Representative Gas Chromatogram of Human Colostrum (TMS Derivatives)

peak 1: glucose & fructose, 2: inositol, 3: lactose
conditions: 2% OV-17 (1.8 m×4 mm *i.d.*), 210°, N_2 90 ml/min

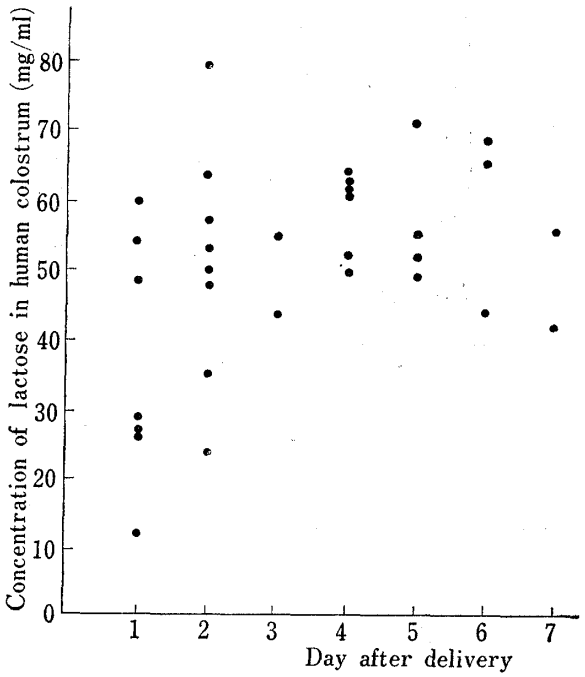

Fig. 4. Correlation between Concentration of Lactose in Human Colostrum and Day after Delivery

Table V. Carbohydrates in Normal Human Blood Plasma after 12 hr Starvation

Subject (initial)	Age (yrs.)	Sex	Sucrose (μg/ml)	Maltose (μg/ml)	Lactose (μg/ml)	Glucose (μg/ml)
A.B	1/3	M	0.82	0.48	0.30	915
S.H	24	M	0.22	1.03	N.D.	807
T.N	24	M	0.05	1.45	N.D.	760

Subject (initial)	Age (yrs.)	Sex	Sucrose (μg/ml)	Maltose (μg/ml)	Lactose (μg/ml)	Glucose (μg/mg)
K.M	24	M	0.11	1.32	N.D.	760
H.N	25	M	0.30	0.64	0.56	845
K.K	25	M	0.05	1.25	0.34	868
M.B	26	F	0.25	0.33	0.65	778
Y.H	26	M	0.21	1.32	0.24	880
T.I	26	M	N.D.	2.12	N.D.	836
H.K	27	M	0.07	1.03	0.31	744
M.Y	28	M	0.03	1.69	0.20	713
Y.S	28	F	0.30	1.94	0.58	790
F.B	30	M	0.40	0.50	0.29	861
S.K	33	F	0.06	0.92	0.23	900
Average			0.21	1.14	0.26	818
S.E			±0.06	±0.15	±0.12	±17

N.D.: detectable but not determined

Table VI. Carbohydrates in Blood Serum of Lying-in Women after 12 hr Starvation

Subject (initial)	Age (yrs.)	Number of delivery	Day after delivery	Sucrose (μg/ml)	Maltose (μg/ml)	Lactose (μg/ml)	Glucose (μg/ml)
F.S	22	1	5	0.14	0.94	11.4	1280
Y.I	23	1	5	0.12	0.66	14.2	824
T.S	24	1	6	0.32	2.52	14.8	1110
M.O	26	1	4	0.14	0.68	19.6	432
N.S	26	1	5	0.14	0.80	33.8	775
M.K	27	1	6	0.06	0.68	10.6	1490
S.H	27	2	6	0.06	0.35	8.76	869
Y.I	28	2	4	0.15	0.52	11.4	1210
S.N	28	2	5	0.13	0.44	12.8	923
S.M	28	2	6	0.17	0.78	15.4	892
Y.M	29	1	7	N.D.	0.63	6.46	246
H.O	30	3	5	N.D.	0.67	29.4	880
K.Y	32	1	7	0.10	2.47	9.00	1560
Average				0.12	0.93	15.2	961
S.E				±0.02	±0.21	±2.24	±104

N.D.: detectable but not determined

Table VII. Carbohydrates in Blood Serum of Diabetics after 12 hr Starvation

Subject (initial)	Age (yrs.)	Sex	Sucrose (μg/ml)	Maltose (μg/ml)	Lactose (μg/ml)	Glucose (μg/ml)
H.T	42	F	0.09	0.41	0.48	1540
T.H	—	F	0.08	0.32	N.D.	2110
H.N	46	F	0.08	0.83	0.42	1330
S.Y	31	M	0.13	0.76	0.70	676
R.K	70	M	0.14	0.76	2.14	1800
K.T	67	M	0.19	0.54	0.29	1090
S.S	—	M	0.50	0.68	1.06	1110
A.T	—	M	0.08	0.38	0.27	1190
Y.O	59	M	0.09	0.78	0.36	1140
U.H	53	M	0.07	1.87	0.46	2250
I.K	—	M	0.16	0.72	0.54	1960
K.H	51	M	0.12	0.27	N.D.	732
M.S	—	M	0.09	0.80	1.21	1140
Average			0.14	0.70	0.61	1390
S.E			±0.03	±0.11	±0.16	±140

N.D.: detectable but not determined

Table VIII. Carbohydrates in Blood Serum of Patients after 12 hr Starvation

Subject (initial)	Sex	Age (yrs.)	Disease	Sucrose (μg/ml)	Maltose (μg/ml)	Lactose (μg/ml)	Glucose (μg/ml)
M.F	F	1	jaundice	0.19	1.23	0.82	806
K.S	M	1	harelip	0.19	0.78	0.78	902
H.S	F	7	epilepsy	0.21	0.74	0.64	844
K.Y	M	2	mucopolysaccharidosis	0.14	0.43	0.60	1164
T.A	M	29	gastric cancer	0.26	2.90	0.46	1088
N.K	M	—	renal disorder	0.13	1.80	0.78	92
A.K	F	32	basedow's disease	0.15	0.46	0.50	903
Y.K	F	50	collagene disease	0.21	0.35	0.25	1010

Table IX. Concentration of Lactose in Human Colostrum

Subject (initial)	Age (yrs.)	Number of delivery	Day after delivery	Concentration (mg/ml)	Average (mg/ml)
F.S	22	1	4	63.0	
Y.I	23	1	5	70.2	
T.T	23	1	4	51.4	
M.S	24	1	2	52.5	
K.M	24	1	3	43.6	
K.S	25	1	1	26.1	49.1
H.M	26	1	1	27.9	
M.O	26	1	4	61.1	
N.S	26	1	5	48.5	
T.K	27	1	2	34.5	
Y.I	27	1	4	61.2	
N.U	26	2	1	26.0	
K.N	26	2	2	78.4	
S.S	26	2	6	64.9	
Y.I	28	2	4	49.7	
S.N	28	2	5	54.1	
S.M	28	2	6	43.6	52.6
M.W	29	2	7	41.4	
Y.K	29	2	4	60.0	
Y.M	33	2	3	54.0	
Y.S	37	2	1	59.0	
S.M	27	2	1	47.6	
K.O	26	3	1	54.0	
N.I	27	3	1	11.1	
K.O	28	3	2	62.6	45.8
H.O	30	3	5	50.9	
Y.T	33	3	2	48.9	
M.H	37	3	2	47.1	
Average				49.8	

Discussion

As shown in Table I, the present method is accurate enough for quantitative analysis of disaccharides in clinical materials.

Sweeley, *et al.*[10] inactivated urease and contaminous transfructosylase by boiling the digest after urease treatment. However, the heating should be avoided, since the pH of urease-treated urine is about 9—10 and disaccharides containing (1→4) or (1→3) linkages are unstable to alkaline treatment.

For the analysis of lactose in human colostrum, removal of lipoid substances, deproteinization and desalt were not required, because lactose occurs in milk in large amount. The

10) C.C. Sweeley, W.W. Wells and R. Bentley, "Methods in Enzymology," Vol. VIII, 1966, p. 95.

average content of lactose in colostrums of 28 healthy women within one week after delivery was 49.8 mg/ml, which is the similar value as the reported ones, 58 mg/g by Saito, *et al.*[11] and 53 mg/ml by Macy, *et al.*[12] Lactulose produces lactitol by treatment with $NaBH_4$,[1] however, as its content is quite low, if present,[13] it will not affect the lactose content we measured.

Generally, disaccharides found in urine come from exogeneous origins except lactose whose concentration in the urine increases markedly during the later stage of pregnancy and in the puerperium, and also except sucrose abnormally synthesized in the human organism *de novo*.[14] Therefore, excretion of disaccharides in urine will mainly depend upon dietary intake. The average concentration of sucrose in urine of 14 healthy men after 12 hr starvation was 102 μg/ml which is rather higher than the reported range of 0—50 μg/ml in normal state,[15] indicating differences in diet and digestive capacity between Japanese and European. However, the range of urinary lactose was 1.13—23.8 μg/ml, which is within the published one, 0—100 μg/ml.[15]

Though the other clinically important disaccharides in urine, such as maltose and isomaltose, were not determined in this work owing to interference of large amount of sucrose, if necessary, the use of borate complex of carbohydrates would be effective. In our experiments, sucrose and α,α-trehalose did not form borate complex, while lactose, maltose, isomaltose, milibiose, gentiobiose and their corresponding disaccharide alditols formed the borate complexes. Actually, the separation of minute amount of urinary lactose and maltose from sucrose was performed by passing the sample through a column of QAE-Sephadex A-25 (borate form, 0.8×3 cm) after deionization by means of Amberlite CG-120 (H^+) and Amberlite CG-4B (CH_3COO^-), leaving sucrose in the effluent. The disaccharides adsorbed were eluted from the column with 5 ml of 0.1 N trifluoroacetic acid.

Many efforts for detecting disaccharides in the blood after ingestion of disaccharide or disaccharide-containing diets have been done in vain, excepting Haworth, *et al.*[16] detected lactose in one of 18 children and one of 10 premature babies, and sucrose in 4 of 22 children. Recently, Jolley, and Freeman, *et al.*[5] detected only sucrose in human blood serum by using a carbohydrate autoanalyzer. However, as far as our knowledge, detection of maltose in the human blood has never been reported. In this investigation, we tentatively identified maltose as well as sucrose and lactose in the blood of normal persons, lying-in women, diabetics and the other patients and further proved that the concentrations of the three disaccharides in the blood in the fasting state were generally low (*ca.* 1 μg/ml). The average content of lactose in the blood of lying-in women was abnormally high level (15.2 μg/ml) as much as about 60 times than that of normal persons and about 25 times than that of diabetics, exceeding the maximal blood level of lactose during lactose tolerance tests (several μg/ml after 50 g lactose).[17]

Acknowledgement We are grateful to Dr. S. Yoshiue, Ome City Hospital, Dr. H. Murooka, the Second Hospital of Japanese Medical University, and Dr. M. Kitamura, Yokohama Branch of Japan Red Cross Hospital, for their cooperation of collecting blood and colostrum. We also wish to thank Mr. I. Matsunaga for his gift of 3-O-methyl-α-D-glucose.

This work was supported partially by a grant from the Iatrochemical Foundation to which the author's thanks are due.

11) K. Saito, E. Furuichi, S. Kondo, G. Kawanishi, I. Nishikawa, H. Nakazato, Y. Noguchi, T. Doi, A. Noguchi and S. Shingo, *Report of Research Laboratory of Snow Brand Milk Products Co., Ltd.*, No. 69 (1965).
12) C. Long (ed), "Biochemists' Handbook," E. & F.N. Spon Ltd., London, 1961, p. 902.
13) S. Adachi, *Nature*, **181**, 840 (1958); S. Adachi, *Nippon Nôgikagaku Kaishi*, **32**, 802 (1958).
14) E.L. Rosenfeld, W.K. Gorodezki and N.A. Zarubina, *Clin. Chem. Acta*, **24**, 469 (1969).
15) C. Long (ed.), "Biochemists' Handbook," E. & F.N. Spon Ltd., London, 1961, p. 923.
16) J.C. Haworth and M.S. Macdonald, *Arch. Dis. Childh.*, **32**, 417 (1957); J.C. Haworth, *ibid.*, **35**, 552 (1960).
17) H. Nakamura and Z. Tamura, *Chem. Pharm. Bull.* (Tokyo), **18**, 2366 (1970).