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SERUM GLYCOPROTEINS IN SCHIZOPHRENIA*†

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ABSTRACT

A comparative investigation of the individual protein-bound carbohydrate components and the distribution of carbohydrates in the electrophoretic glycoprotein fractions was carried out in sera from 20 normal subjects and 30 schizophrenic patients matched for age and sex. The mean concentration of each of the protein-bound carbohydrate components was significantly elevated in schizophrenics. The electrophoretic patterns for serum glycoprotein showed increases in alpha-2 and beta globulins in schizophrenics. The serum glycoproteins contained glucose and L-arabinose, in addition to mannose, galactose, fucose, sialic acid, and a trace of xylose. The identity of glucose and arabinose was confirmed by g.l.c.–electron-impact mass spectrometry and by specific enzymic reactions. The contents of glucose and arabinose were higher in serum glycoproteins from schizophrenic patients. This elevation of serum glycoprotein paralleled serum glycosaminoglycan elevation previously reported by us, but was opposite to decrease of urinary glycoprotein in schizophrenics.

INTRODUCTION

Disturbances in carbohydrate metabolism and plasma-protein abnormalities in schizophrenic patients have long been known, and have been discussed in a number of reviews[1–5]. The relationship of carbohydrate metabolism to psychotic behavior is suggested by several observations. Among these are: presence of protein-bound carbohydrates (glycoproteins and glycosaminoglycans) in the brain as components of the blood–brain barrier and neuronal receptor-sites, and for binding of biogenic amines[6]; mental dysfunctions in abnormal metabolism of these macromolecules[1,6]; abnormal blood–brain permeability in mental patients[7]; absence of rheumatoid

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arthritis in schizophrenics[8]; changes in brain mucoids in learning and training[9]; and behavior improvements by intrathecal injection of the enzyme hyaluronidase in excited patients[10], in patients with thought-block, and in those with senile arteriosclerotic behavior-disorders[11]. The presence of abnormal leukocytes in schizophrenics[12] may contribute to alterations in glycoproteins and glycosaminoglycans in the sera of these patients.

Because of the aforementioned relationship of carbohydrate metabolism to mental illness, we have been studying glycoproteins and glycosaminoglycans in body fluids of schizophrenics. In our previous papers[13-16], we reported decreased excretion of glycoproteins and glycosaminoglycans and a decreased ratio of high-molecular-weight glycosaminoglycans to their low-molecular-weight degradation products, together with other changes in the urine of schizophrenic patients. In a recent paper[17], we reported elevation of serum glycosaminoglycan levels in schizophrenics. As in several disease-states, serum glycoproteins parallel the serum glycosaminoglycan levels, and furthermore, the abnormal leukocytes[12] reported in schizophrenics may contribute to serum glycoproteins, we decided to study these serum glycoproteins. This paper describes a comparative investigation on serum glycoproteins in 30 paranoid-schizophrenic patients matched for age and sex with 20 normal control subjects.

RESULTS AND DISCUSSION

Paper chromatograms of the deionized hydrolyzate of the serum glycoproteins from both normal and schizophrenic patients revealed the neutral sugars: fucose, glucose, and galactose, and a spot having the R_F value common to mannose and arabinose. The material eluted from a strip corresponding to the mannose + arabinose spot on a preparative paper chromatogram, when analyzed by g.l.c. as the peracetylated aldononitriles, showed mainly mannose, with arabinose as the minor component. Enzymic analysis of this isolated material by use of L-arabinose isomerase and L-*erythro*-pentulose kinase, which are highly specific for their substrates, further confirmed the presence of L-arabinose. The former enzyme converts L-arabinose into L-*erythro*-pentulose, which then acts as a substrate for the latter enzyme[18]. Gas chromatography of the peracetylated nitriles of the neutral sugars, and the anhydro sugars from the 2-amino-2-deoxyhexoses[19] present in the deaminated and deionized hydrolyzates of serum glycoproteins from both normal and schizophrenic subjects, showed the presence of fucose, arabinose, 2-amino-2-deoxyglucose, 2-amino-2-deoxygalactose, mannose, glucose, and galactose (Fig. 1). Most of the samples also contained a trace of xylose. The quantitative values for serum glycoproteins of normal and schizophrenic subjects are presented in Table I. All of the individual carbohydrate components of serum glycoproteins were elevated in schizophrenic patients. The g.l.c.–electron-impact mass-spectrometric analysis of the aldononitrile peracetates of the mixture of the neutral sugars[20,21] further verified the presence of arabinose and glucose in the serum glycoprotein hydrolyzates, together with fucose, mannose, and galactose.

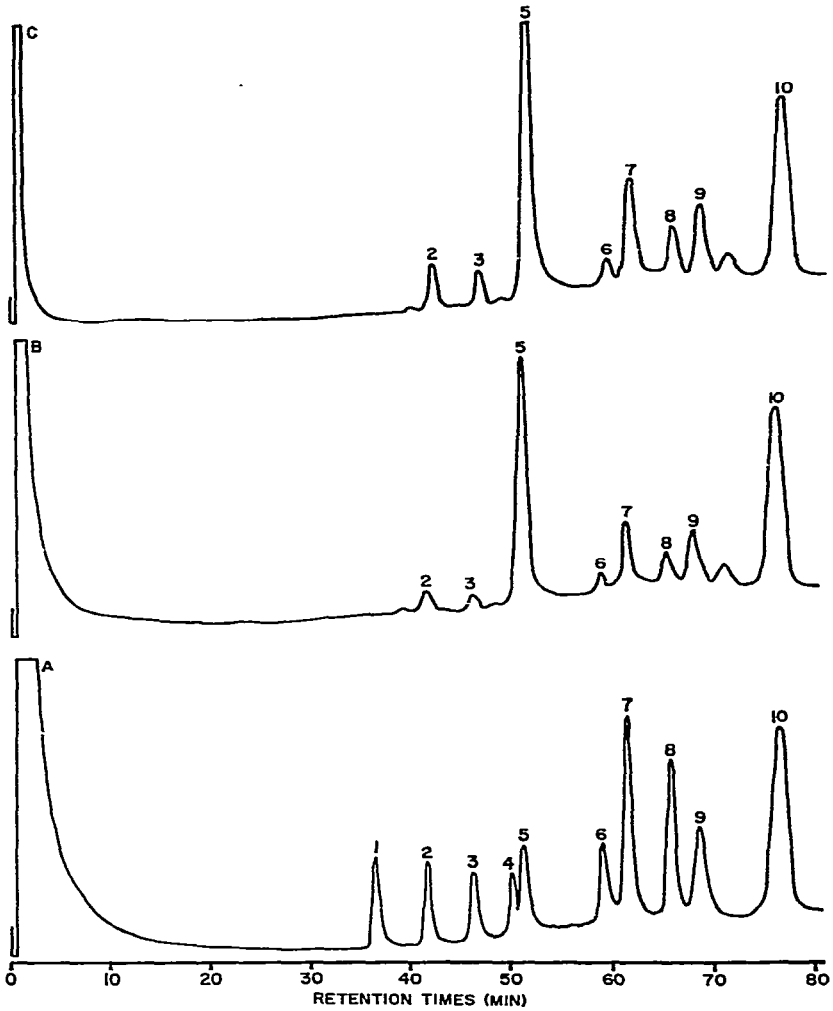

Fig. 1. Gas chromatograms of the peracetylated aldononitriles of standard, neutral sugars and deaminated 2-amino-2-deoxyhexoses (A), of peracetylated aldononitriles of neutral sugars and deaminated 2-amino-2-deoxyhexoses from normal serum glycoprotein (B), and from serum glycoprotein of schizophrenics (C). The peaks are as follows: 1, rhamnose; 2, fucose; 3, arabinose; 4, xylose; 5, 2-amino-2-deoxyglucose; 6, 2-amino-2-deoxygalactose; 7, mannose; 8, glucose; 9, galactose; and 10, *myo*-inositol. The small peak between 9 and 10 was not identified.

Electrophoresis on cellulose acetate separated serum glycoproteins into alpha-1, alpha-2, beta, and gamma globulins. Analysis of the carbohydrate distribution in these separated globulin-fractions by the periodic acid–Schiff reaction followed by a densitometric scan of the stained electrophoretic pattern gave the values recorded in Table I. The content of alpha-2 and beta globulins was elevated in sera from the schizophrenic patients. The carbohydrate compositions of the glycoproteins from patients on medication and off medication were not significantly different (Table II).

TABLE I

SERUM PROTEIN-BOUND CARBOHYDRATE COMPONENTS (mg/100 mL) AND SERUM GLOBULINS (% OF TOTAL) IN NORMAL SUBJECTS AND SCHIZOPHRENIC PATIENTS

Component	*Normals* (N = *20*)		*Schizophrenics* (N = *30*)		t-	P
	Range	*Mean ±S.D.*	*Range*	*Mean ±S.D.*	*Value*	
Fucose	2.70–5.64	4.11 ±1.60	4.26–11.60	8.57 ±1.92	8.92	<0.001
Arabinose	1.51–2.84	1.95 ±0.87	1.96–3.60	2.72 ±1.40	2.33	<0.05
Xylose	Trace (not calculated)		Trace (not calculated)			
Mannose	28.45–73.17	44.95 ±7.01	45.05–89.40	73.35 ±9.61	12.09	<0.001
Galactose	32.44–74.23	43.17 ±9.67	48.57–77.84	55.95 ±8.66	4.77	<0.001
Glucose	2.95–5.4	4.34 ±0.79	5.11–8.77	6.64 ±1.24	8.21	<0.001
Sialic acid	53.59–77.53	65.61 ±7.80	58.44–92.70	72.49 ±8.07	3.01	<0.01
Total hexose	65.80–105.50	93.46 ±14.99	88.20–143.50	115.49 ±15.78	4.99	<0.001
2-Amino-2-deoxy-hexose	59.20–90.42	81.93 ±17.93	76.42–110.80	94.73 ±18.87	2.42	0.02
α_1-Globulin	8.20–13.60	11.22 ±2.14	7.80–13.30	10.96 ±2.91	0.37	[a]
α_2-Globulin	38.30–47.10	41.08 ±4.20	39.10–49.40	43.55 ±4.13	2.05	0.05
β-Globulin	27.20–33.10	30.08 ±3.90	27.60–38.00	33.70 ±4.34	3.07	<0.01
γ-Globulin	10.90–16.10	14.28 ±2.94	9.80–18.80	12.82 ±4.21	1.44	[a]

[a]Not significant.

TABLE II

SERUM PROTEIN-BOUND CARBOHYDRATE COMPONENTS (mg/100 mL) IN SCHIZOPHRENIC PATIENTS ON MEDICATION AND OFF MEDICATION

Component	*Medicated patients* (N = *11*) *Mean ±S.D.*	*Non-medicated patients* (N = *19*) *Mean ±S.D.*	t-*Value*	P
Fucose	8.49 ±1.95	8.73 ±1.88	0.33	[a]
Arabinose	2.69 ±1.37	2.93 ±1.41	1.44	[a]
Xylose	Trace	Trace		
Mannose	75.14 ±9.46	71.01 ±8.96	1.17	[a]
Galactose	52.76 ±8.94	55.89 ±7.97	0.96	[a]
Glucose	6.51 ±1.36	6.62 ±1.28	0.21	[a]
Sialic acid	73.54 ±8.12	70.94 ±8.09	0.84	[a]
2-Amino-2-deoxyhexose	91.24 ±17.96	95.91 ±17.72	0.68	[a]

[a]Not significant.

The molar ratios of the principal carbohydrate components are given in Table III. The molar ratios of fucose/sialic acid, fucose/hexose, fucose/hexosamine, and hexose/hexosamine were increased in schizophrenics. However, the molar ratio of pentose/hexosamine remained unchanged in the patients. These increases of molar ratios of the carbohydrate constituents further suggested chemical differences of the glycoproteins in schizophrenic patients.

The carbohydrates present in serum glycoproteins are covalently linked to proteins. Therefore, quantitative measurement of the protein-bound carbohydrates provides an index of the gross picture of the changes in serum glycoprotein. The serum

TABLE III

MOLAR RATIOS OF PROTEIN-BOUND CARBOHYDRATE COMPONENTS IN NORMAL SUBJECTS AND SCHIZOPHRENIC PATIENTS

Carbohydrate components	*Normals* (N = *20*) *Range*	*Mean*	*Schizophrenics* (N = *30*) *Range*	*Mean*
Fucose/sialic acid	0.09–0.13	0.12	0.13–0.24	0.22
Fucose/hexose	0.04–0.06	0.05	0.05–0.09	0.08
Fucose/hexosamine	0.05–0.07	0.06	0.06–0.11	0.10
Hexose/hexosamine	1.10–1.16	1.13	1.15–1.28	1.21
Pentose/hexosamine	0.02–0.02	0.02	0.02–0.02	0.02

glycoproteins contain hexoses, hexosamine, and sialic acid (in descending order of concentration)[22]. Only two hexoses, namely, mannose and galactose, have been reported in serum glycoproteins. Recently, the presence of glucose[23] has been reported, and our study has also shown arabinose to be present. Both glucose and arabinose, like the other carbohydrate components, are elevated in schizophrenic patients.

The precipitate obtained upon adding 95% ethanol to serum contains glucose, which decreases during three subsequent washes with ethanol. Apparently, some of the free glucose of serum becomes entrapped during the initial precipitation step, and is lost upon washing with ethanol. The glucose and arabinose present in the serum glycoprotein after the third ethanolic wash constitute the protein-bound neutral sugars, and are not lost, even after repeated and prolonged dialysis against water.

The molar ratios of the carbohydrate constituents are altered in various disease states. Of the carbohydrate components, fucose is the most variable and its proportion varies with respect to other components in various neoplastic and inflammatory conditions. The fucose/hexosamine ratio is constant in normal subjects. The fucose/hexose ratio is constant in single individuals, but varies in the alpha and beta globulin fractions in different individuals[24]. These alterations in the molar ratios of the various carbohydrate components reflect the changes in glycoprotein from the sera of the schizophrenics.

Numerous individual, unrelated glycoproteins are present in serum, and the concentration of each of these may vary independently of the concentrations of others[22]. From this observation, it appears that the individual glycoproteins may have different sources and differences in significance. The majority of serum glycoproteins are of hepatic origin, some are from endocrine organs, and a few may appear as a result of tissue injury.

The elevation of serum glycoproteins is not specific to schizophrenia; it is observed in several inflammatory conditions and in cancer[22]. Several workers have reported alterations in blood proteins in schizophrenics[2–5]. This significant elevation of protein-bound carbohydrates, and increases in the alpha-2 and beta globulin fractions in schizophrenics observed here, may be related to the changes in blood

proteins reported by other workers.

Schizophrenics are believed to be under constant stress. As a physiological response to this stress, the acute-phase proteins of plasma may become elevated[3]. Some of the changes in serum glycoprotein in schizophrenics may be attributable to the physiological response of their bodies to psychotic tension and excitation.

EXPERIMENTAL

Reagents and standards. — Standard sugars were obtained from Sigma Chemical Co., St. Louis, MO. The ion-exchange resins, AG 1-X2 (Cl^- form, 200–400 mesh) and AG 50W-X8 (H^+, 100–200 mesh) were obtained from BioRad Laboratories, Richmond, CA. The ion-exchange resin AG 1-X2 (HCO_3^- form) was prepared from AG 1-X2 (Cl^-) resin packed in a column and sequentially washed with 2M sodium hydroxide, distilled water, and then with 2M sodium hydrogencarbonate and distilled water.

Selection of control and experimental groups. — The control and experimental groups consisted of white males of ages between 22 and 55 years. The normal group consisted of 20 healthy hospital employees. The experimental group included 30 patients selected from those newly admitted (2 patients) and those with prolonged illness. Out of these patients, 11 received ataractic drugs, 19 were off medication. Schizophrenia was diagnosed in these patients initially according to the American Psychiatric Association's Diagnostic and Statistical Manual of Mental Disorders (DSMII). To eliminate overdiagnosis, these patients were then screened by the more rigorous and restrictive research-diagnostic criteria of Feighner *et al.*[25], which considerably decreased the number of patients previously labeled as schizophrenics. The differences between the means for the normal and test groups were evaluated by application of the Students' *t*-test.

Isolation of neutral and amino sugars (as anhydro sugars). — The following steps were employed:

(*a*) To 0.25 mL of serum contained in a 10-mL glass ampoule was added 2.7 mL of 95% ethanol. Following mixing and after 5 min, the ampoule was centrifuged for 15 min at 500 r.p.m. and the supernatant solution was discarded.

(*b*) The precipitate was washed three times with 3 mL of 95% ethanol, with vortexing and centrifugation. The precipitate was dried in a vacuum desiccator overnight.

(*c*) By using vortexing, the solid in the ampoule was dissolved in 0.5 mL of 0.1M sodium hydroxide and 1 mL of distilled water. To this solution was added 0.15 mL of conc. hydrochloric acid. The solution was deaerated with nitrogen for 5 min, and the ampoule was sealed tightly and heated for 4 h at 100° in an oven.

(*d*) After cooling the ampoule to room temperature for 5–10 min, the tip was carefully broken off and 0.2 mL of a freshly prepared solution (500 mg/mL) of sodium nitrite was added. The tip of the vial was quickly resealed, keeping the flame away from the contents of the vial. The contents were vortexed for 25–30 min during

which time deamination of 2-amino-2-deoxyhexoses to anhydro sugars was completed.

(*e*) The ampoule was broken and the pH of the solution was adjusted to 5–6 with a pinch of AG 1-X2 (HCO_3^-) resin. The solution was passed through a tandem arrangement of two columns; the upper column (1 × 4 cm) was packed with AG 50W-X8 (H^+, 100–200 mesh) resin and was drained into the lower column (1 × 5 cm) packed with AG 1-X2 (HCO_3^-) resin. The eluate (~70–75 mL) and the water washings were collected, evaporated at 40–45° to low volume in a rotary evaporator, and transfered into a 10-mL glass ampoule. To this solution was added an appropriate amount of *myo*-inositol (internal standard, 1 mg/mL), and the mixture was lyophilized.

Derivatization for gas chromatography. — The residue in the ampoule was dried for 10 min in a vacuum desiccator. The aldoses and the 2,5-anhydro sugars were converted into their aldononitrile peracetates as described previously[19]. The quantities of various reagents used were: hydroxylamine hydrochloride, 3–5 mg; pyridine, 5 drops; and acetic anhydride, 15 drops. The derivatized residue was dissolved in 100 μL of chloroform and 4–6 μL was subjected to gas chromatography.

Preparation of standards. — A standard solution (4 mL) was prepared that contained 8 mg each of L-rhamnose (8.8772 mg of L-rhamnose · H_2O), L-fucose, L-arabinose, and D-xylose; 16 mg each of D-mannose, D-glucose, D-galactose, 2-amino-2-deoxy-D-glucose, and 2-amino-2-deoxy-D-galactose (19.2596 mg of their hydrochlorides). This solution (0.5 mL) was freeze dried and subjected to the steps (*c*)–(*e*) as described for serum glycoproteins. The amount of sodium nitrite (500 mg/mL) added was 0.3 mL, and the internal standard solution (1 mg/mL) was added before the mixture was lyophilized. The quantities of reagents used for derivatization were: hydroxylamine hydrochloride, 12–14 mg; pyridine, 15 drops; and acetic anhydride, 45 drops. The derivatized residue was dissolved in 2 mL of chloroform and 2–4 μL was subjected to gas chromatography.

Gas chromatography. — The mixture of aldononitrile acetates of neutral aldoses and 2,5-anhydro hexoses (from deamination of 2-amino-2-deoxyhexoses) was resolved on a Series 1200 Varian Aerograph equipped with a flame-ionization detector and a 3380A Hewlett–Packard integrator. A stainless-steel column (3 m × 0.32 cm) packed with 3% of poly(neopentylglycol succinate) on Gas Chrom W, AW (100–120 mesh) was used. The injector and detector temperatures were 130° and 230°, respectively. The sugars were eluted with temperature programming of 1°/min from 130 to 195° with nitrogen as the carrier gas at a flow rate of 45 mL/min.

Electrophoresis on cellulose acetate. — Electrophoretic separation of serum glycoproteins into α_1, α_2, β, and γ globulins in a microzone cell, periodic–Schiff staining, and the densitometric scanning of the electrophoretic patterns were performed according to the Beckman Microzone Methods Manual (Beckman Instruments, Palo Alto, CA).

Analytical and preparative paper-chromatography of neutral sugars from serum glycoproteins. — The neutral sugars were isolated by the aforementioned procedure, except that the deamination step (*d*) was omitted and a longer column of AG 50W-X8 (H^+) was used to ensure complete removal of the 2-amino-2-deoxyhexoses. The

chromatography was performed with two overnight ascents in 6:4:3 (v/v) butanol–pyridine–water followed by a third overnight ascent in 12:5:4 (v/v) ethyl acetate–pyridine–water as described elsewhere[26].

Enzymic analysis of L-*arabinose.* — The enzymes L-arabinose isomerase and L-*erythro*-pentulose kinase, prepared from *Escherchia coli* mutants, were gifts from Dr. Nancy Lee, University of California at Santa Barbara. The enzymic analysis was effected as described elsewhere[18].

Electron-impact mass spectrometry. — The g.l.c.–mass-spectrometric work was performed at the Virginia State University. The spectra were recorded on a Varian–MAT Model 112 GC-MS instrument equipped with a Spectrosystem 100 computer, with an inlet temperature of 260°, an ionization potential of 80 eV, and an ion-source temperature of 260°. The instrument was fitted with a glass column (1.6 m × 0.32 cm) packed with a mixture of 3% of OV-225 and 2.75% of tetramethylcyclobutanediol succinate on Supelcoport (80–100 mesh). Helium was used as the carrier gas at a flow rate of 27 mL/min.

Sialic acid analysis. — The sialic contents of the serum glycoproteins were determined according to Winzler[27].

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