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# HEXOSAMINES OF SEROMUCOID<sup>1</sup>

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A renewal of interest in seromucoid, or the acid mucoprotein of human serum, was initiated by Winzler, Devor, Mehl, and Smyth in 1948 (1). Since that time many studies of the significance of this fraction have been published (2-4). It is now well established that this carbohydrate-rich protein fraction is increased in the serum in a wide variety of disease states, including acute and chronic inflammation and metastatic malignancies. Decreased levels have been found in nephrosis and cirrhosis. These variations of seromucoid in disease have recently been reviewed by Greenspan (5) and by Winzler (6). In general, it has been postulated that the seromucoid elevations are in some way related to destruction and/or proliferation of connective tissue ground substance, and the reduced levels in nephrosis and cirrhosis represent urinary loss and defective formation of seromucoid, respectively.

While the recent literature is replete with studies of the variation of total seromucoid in disease (2-6), there is considerably less information on variations of the carbohydrate components within this fraction which is very probably quite heterogeneous. There appear to be at least three electrophoretically distinct components in this fraction (7), and there is evidence that the carbohydrate to protein ratio of seromucoid may vary in different diseases (2, 8). If the circulating seromucoid is indeed related to the destructive and/or proliferative changes occurring in the connective tissue ground substance, then qualitative differences in this serum fraction in these clinically distinct diseases might indicate some basic difference in the pathologic process at the tissue level. Both connective tissue ground substance and seromucoid are rich in hexosamine. In the case of ground substance a good deal of information has been accumulated about the mucopolysaccharide compo-

sition of various tissues (9). The chondroitin sulfates and the non-sulfated chondroitin contain galactosamine, while hyaluronic acid contains only glucosamine. Both glucosamine and galactosamine have been found in seromucoid (10, 11) but little is presently known of the ratio of these two hexosamines in this fraction in normal subjects or in disease states where the total quantity of seromucoid is altered. The purpose of the present study, therefore, was to determine the quantities of glucosamine and galactosamine in seromucoid in normal subjects and in certain disease states.

## METHODS

Perchloric acid was added to 10 ml. of serum diluted to 20 ml. with water, to a final concentration of 0.66 N HClO<sub>4</sub>, and the filtrate obtained as described by Winzler and co-workers (1). This filtrate was dialyzed against running tap water in the cold room (4° C.) for four to six days, and then lyophilized. The powdered seromucoid was hydrolyzed with 2.0 N HCl for 15 hours in a steam bath. After cooling and filtering, the hydrolysate was placed on a small Dowex 50 ion exchange column from which hexosamine was eluted and determined as described by Boas (12) to give the seromucoid hexosamine. An aliquot of this hexosamine eluate containing approximately 150 micrograms of hexosamine was further separated into glucosamine and galactosamine by a modification (13) of the method of Gardell (14). This involves elution of the hexosamines from a 30 by 0.8 cm. column of Dowex 50 (hydrogen form) using the concentration gradient elution principle (0.6 N HCl in the reservoir with a 500 ml. mixing flask of water) and collecting the eluate with an automatic fraction collector. The hexosamine content of each tube of eluate was determined and plotted as a curve. There was adequate separation of the two hexosamines in all cases. The areas under each peak were calculated by planimetry if there was any overlap of the curves and from these values and the total seromucoid hexosamine both the relative and absolute quantities of each hexosamine were calculated.

When serum protein fractions other than seromucoid were studied, *i.e.*, whole serum protein and the mucoid fractions of Dische and Osnos (15), they were hydrolyzed in 2.0 N HCl and subsequently handled in the same fashion as the seromucoid hydrolysate described above.

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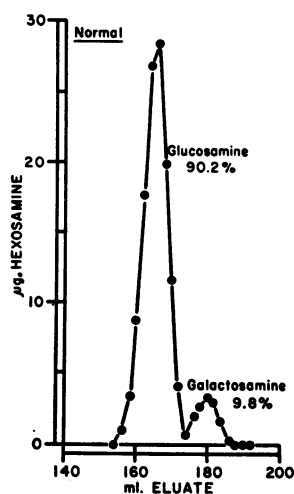


FIG. 1. SEPARATION OF HEXOSAMINES OF NORMAL SEROMUCOID

#### RESULTS

Figure 1 shows the separation of glucosamine and galactosamine in normal seromucoid. Values for the normal group are shown in Table I. The glucosamine content ranged from 86.3 to 96.8 per cent of the total seromucoid hexosamine, with a mean of 91 per cent. Galactosamine made up the remainder. The total recovery of hexosamine in the two peaks invariably approximated very closely (within  $\pm 4$  per cent) the total hexosamine applied to the column. No other peaks were ever noted. The ratio of galactosamine to glucosamine of the normal subjects ranged from 0.03 to 0.16. The total seromucoid hexosamine in these subjects is in the general range of values expected from the findings of Winzler and co-workers (1) that there are about 30 milligrams seromucoid per 100 ml. of serum, 12 per cent of which is hexosamine.

Although the number of patients with connective tissue diseases is limited, these subjects presented a classical example of each disease in its full-blown state of activity. These data are presented in Table I. These subjects in general had some increase in total seromucoid hexosamine but did not demonstrate any significant deviation from the normal galactosamine to glucosamine ratio. In four of these subjects the total serum protein-bound hexosamine was separated into galactosamine and glucosamine and this too did not differ

from the normal ratio of 90 to 95 per cent glucosamine and 5 to 10 per cent galactosamine.

The most striking abnormalities of seromucoid galactosamine to glucosamine ratio were found in the three patients with nephrotic stage of glomerulonephritis. Each of these patients was an adult who initially presented the complete clinical picture of this disease state. Pertinent clinical data are in Table II, included in which are the data on a patient with histologically proven amyloid nephrosis secondary to tuberculosis of the spine and lungs (patient No. 1 of the miscellaneous group in Table I). The hexosamine separations in two of these patients, M. I. L. and B. E. L., are shown in Figures 2 and 3, respectively, both before and after some clinical response to therapy. These illustrations demonstrate the altered relationship of galactosamine and glucosamine, when compared with the normal in Figure 1, resulting in the elevated galactosamine to glucosamine ratios indicated in Table I. The average value for this ratio in the three patients with the nephrotic phase of glomerulonephritis, including values before and after some response to therapy, was 0.38. These elevated ratios were apparently due to a decrease in seromucoid glucosamine and an actual increase in galactosamine. The severity of the nephrotic state in these three patients with nephritis did not differ significantly from that in the subject with amyloid nephrosis in terms of edema, proteinuria, hypoproteinemia, and hypercholesterolemia; however, the total seromucoid was distinctly elevated in the latter and the striking disproportion of galactosamine and glucosamine found in the other three patients was not evident.

In the heterogeneous miscellaneous disease group were subjects with elevated and reduced levels of total seromucoid hexosamine. Two patients with chronic glomerulonephritis and parathyroid adenoma, respectively, and the two with myxedema had somewhat increased galactosamine to glucosamine ratios while the remainder had essentially normal ratios.

Figure 4 demonstrates that there is only one hexosamine in a mucoid preparation of whole normal serum. This was a fraction prepared by alkali-alcohol treatment of serum as described by Dische and Osnos (15). When this mucoid fraction was hydrolyzed and fractionated, only one peak was found. When standard galactosamine

TABLE I  
Total Serum and Seromuroid Hexosamine and Seromuroid Glucosamine and Galactosamine  
in Normal Subjects and Patients with Various Diseases

SUBJECTS	Total Serum Hexosamine ng/100 ml.	Seromuroid Hexosamine ng/100 ml.	% of Seromuroid Hexosamine as		Seromuroid Hexosamine ng/100 ml.		Ratio Galactos- amine:Glu- cosamine
			Glucosamine	Galactosamine	Glucosamine	Galactosamine	
<b>NORMAL GROUP</b>							
1). H.V.M.		2.94	94.2	5.8	2.77	0.17	0.06
2). W.P.D.		3.09	90.2	9.8	2.79	0.30	0.11
3). H.P.	72.2	1.40	86.3	13.7	1.21	0.19	0.16
4). F.J.O.		3.15					
5). S.R.M.		2.40					
6). F.E.P.	83.1	3.49	95.1	4.9	3.32	0.17	0.05
7). C.H.W.	96.6	5.07	87.2	12.8	4.42	0.65	0.15
8). A.C.		2.62	88.1	11.9	2.31	0.31	0.13
9). R.R.M.	70.1	3.6	96.8	3.2	3.48	.12	0.03
Average		3.08	91.13	8.87	2.90	0.27	0.10
<b>CONNECTIVE TISSUE DISEASES</b>							
1). B.U.R. (Scleroderma)		4.00	88.0	12.0	3.52	0.48	0.14
2). L.E.W. (Scleroderma)	76.0	4.00	88.9	11.1	3.55	0.45	0.13
3). C.A.R. (F.A.M.)		7.58	90.3	9.7	6.84	0.74	0.11
4). M.O.O. (D.L.E.)		3.60	69.2	10.8	3.21	0.39	0.12
5). K.I.G. (Rheum. Arth.)	100.2	3.94	84.6	15.4	3.33	0.61	0.18
6). W.O.R. (Rheum. Arth.)		6.76	96.3	3.7	6.51	0.25	0.04
7). S.I.L. (Rheum. Spond.)		7.04	85.7	14.3	6.03	1.01	0.17
Average		5.27	89.0	11.0	4.71	0.56	0.13
<b>NEPHROTIC SYNDROME</b>							
1). M.I.L. Relapse	97.6	2.60	69.7	30.3	1.81	0.79	0.44
Partial (Steroid) Remission		3.08	76.9	23.1	2.37	0.71	0.30
2). B.E.L. Relapse	100.2	1.30	53.1	46.9	0.69	0.61	0.88
Partial Remission	110.1	2.85	73.2	26.8	2.04	0.76	0.37
3). M.C.H. Relapse	41.3	1.30	72.3	27.7	0.94	0.36	0.38
Partial (Steroid) Remission	65.4	1.57	83.6	16.4	1.31	0.26	0.20
Average		2.12	71.5	28.5	1.54	0.58	0.38
<b>MISCELLANEOUS DISEASES</b>							
1). A.B.M. Amyloid Nephrosis		6.07	85.6	14.4	5.19	0.87	0.17
Terminal Uremia	93.0	7.87	85.3	14.7	6.70	1.17	0.17
2). H.A.R. Acute Nephritis		5.34	92.5	7.5	4.94	0.40	0.08
3). S.M.A. Chr. Nephritis		2.28	81.2	18.8	1.85	0.43	0.23
4). J.O.N. Chr. Nephritis with Osteitis Fibrosa Cystica		5.15	92.0	8.0	4.74	0.41	0.09
5). B.K.E. Parathyroid Adenoma		6.64	83.2	16.8	5.52	1.11	0.20
6). S.K.N. Sarcoid with hypercal- cemia	94.8	8.37	92.4	7.6	7.73	0.64	0.08
7). F.I.T. Cirrhosis	85.1	4.20	96.2	3.8	4.04	0.16	0.04
8). C.O.N. Cirrhosis	23.7	2.40	97.3	2.7	2.34	0.06	0.03
9). H.A.R. "Stiff Man Syndrome"	65.8	2.10	98.5	1.5	2.07	0.03	0.01
10). R.O.W. Diabetes	70.2	1.70	96.8	3.2	1.65	0.05	0.03
11). H.U.W. Hyperthyroidism	101.6	2.19	93.6	6.4	2.05	0.14	0.07
12). C.H.E. Myxedema	53.4		83.7	16.3			0.19
13). F.E.E. Myxedema	75.0	3.29	81.2	18.8	2.57	0.62	0.24
14). L.E.F. Melanosarcoma	114.7		89.6	10.4			0.11
15). T.H.O. Breast Cancer	113.0		94.7	5.3			0.06

was added to an aliquot of this mucoid hydrolysate, two peaks appear. This was found to be true of both fractions I and II, containing 57 and 8 per cent, respectively, of the total serum hexosamine in both normal and nephrotic individuals. This is taken as evidence that glucosamine is the only hexosamine present in these two fractions.

#### DISCUSSION

The observations of this study indicate that, though the seromuroid levels are increased in connective tissue diseases and many other disease

states in the miscellaneous group, there is little change in the galactosamine:glucosamine ratio. This could mean that there is simply an increase in proliferation and/or destruction of the same ground substance components which are normally being synthesized and degraded.

An explanation is needed for the abnormal ratio of galactosamine and glucosamine in the nephrotic phase of glomerulonephritis. While it is not proven it seems likely that the decreased levels of seromuroid glucosamine which were noted in this study and which account for the reduction of total seromuroid hexosamine are most logically attrib-

TABLE II  
Clinical and Laboratory Data on the Nephrotic Patients

Subjects	Clinical Data	Therapy	Cholesterol	TSP	A	G	Urine Protein
M.I.L.	Edematous	0	400	5.0	1.8	3.2	5.3 g/1
	15 lb. wt. loss	40 mg. prednisone daily x 3 weeks	460	5.3	2.3	3.0	3.7 g/1
B.E.L.	Edematous	0	800	4.2	1.1	3.1	7.5 g/1
	25 lb. wt. loss	200 mg. Na diet 4 wks.	480	6.2	2.5	3.7	0
MCH	Edematous	0		3.6	1.0	2.6	15.5 g/1
	13 lb. wt. loss	80 mg. prednisone daily x 3 weeks	538	4.7	1.9	2.8	5 g/1
A.B.N.	Edematous, Azotemia	0	430	3.6	1.0	2.6	15 g/1
	No response to E <sub>x</sub> . Terminal uremia. Died 2 wks. later	200 mg. Na diet 5 weeks	180	3.8	1.3	2.5	

utable to urinary loss of the glucosamine-containing components of seromuroid. This would explain the return toward normal levels when proteinuria was reduced by therapy. These observations are in accord with the findings of Kelley, Good, and Glick (16), who noted decreased total seromuroid levels in nephrosis which promptly increased as proteinuria lessened.

The other abnormality in seromuroid hexosa-

mine in this disease, the elevated galactosamine, is assumed to represent an increase in the galactosamine-containing components of seromuroid. It is perhaps not surprising that in this disease there should be urinary loss with a decreased serum level of one (the glucosamine-containing) component of the heterogeneous seromuroid fraction while there is an actual increase in the serum level of another (the galactosamine-containing)

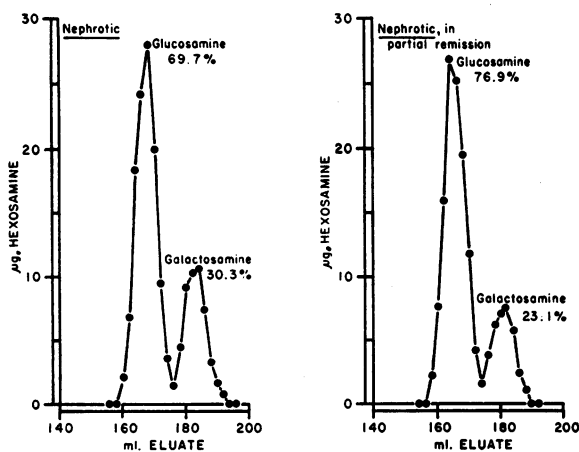


FIG. 2. SEPARATION OF HEXOSAMINES OF SEROMUCOID OF A NEPHROTIC PATIENT (M.I.L.) BEFORE AND AFTER SOME CLINICAL REMISSION

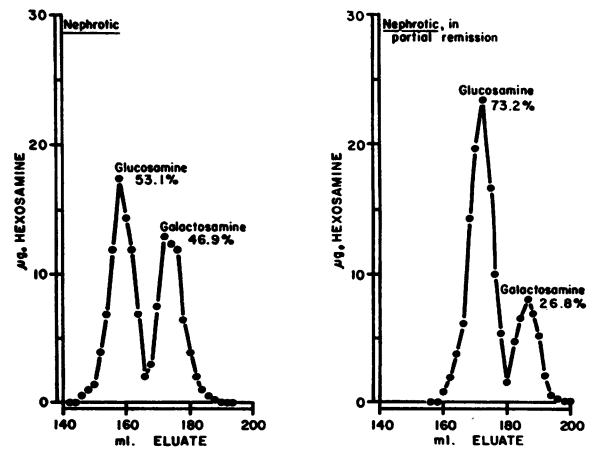


FIG. 3. SEPARATION OF HEXOSAMINES OF SEROMUCOID OF A NEPHROTIC PATIENT (B.E.L.) BEFORE AND AFTER SOME CLINICAL REMISSION

component. There is certainly ample evidence of this same general phenomenon with other serum proteins; notably the striking loss of albumin with hypoalbuminemia along with huge increases in some of the serum lipoprotein fractions. One might infer from this that glucosamine-containing seromuroid is of smaller molecular size than galactosamine-containing seromuroid. The contrast between the low seromuroid levels in the nephrosis of chronic glomerulonephritis and the elevated levels in amyloid nephrosis has been previously noted (5) and remains unexplained. In our patient with amyloidosis the proteinuria was as great as in the other nephrotics. In the former there was an increase in both glucosamine and galactosamine.

Generalizations regarding the values in the miscellaneous group seem inappropriate since there was considerable variation and the numbers of patients with each disease state was quite small. These subjects were included in this study in the process of surveying groups of diseases for abnormalities in seromuroid. One finding which is worthy of further study was the increased galactosamine to glucosamine ratios found in two patients with primary myxedema. This disease has in common with nephrosis the hypercholesterolemia, and perhaps the galactosamine-containing seromuroid is related to the lipid disturbance in these diseases. It is also worth pointing out that in primary myxedema there is, of course, the increase in connective tissue ground substance which may in some way be reflected in the altered galactosamine to glucosamine ratio.

It is of some interest that the galactosamine bound to serum protein does not belong to the mucoid fractions which can be split from the protein readily by mild treatment with alkali. Dische and Osnos, who described this method of liberating mucoid substances from serum protein (15), believe that these two fractions, containing 70 and 15 per cent of the total serum protein bound carbohydrate, represent mucopolysaccharide moieties loosely bound to protein. It is clear from our observations that the hexosamine of both of these two alkali-labile fractions is glucosamine, both in the normal and in the nephrotic. This is at variance with the report that this hexosamine is galactosamine (17) and we have no explanation for this discrepancy, except that the latter authors

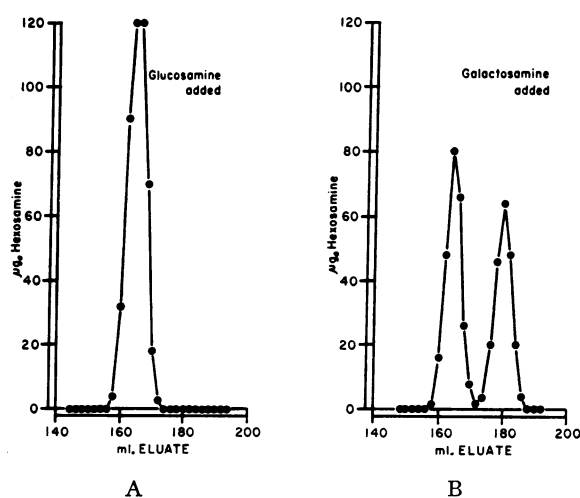


FIG. 4. SEPARATION OF HEXOSAMINE OF NORMAL SERUM MUCOID FRACTION I, WITH GLUCOSAMINE ADDED (A) AND WITH GALACTOSAMINE ADDED (B)

used a different fractionation scheme for the hexosamines.

#### SUMMARY

1. The quantities of glucosamine and galactosamine in seromuroid of seven normal subjects, seven patients with connective tissue diseases, three patients with the nephrotic state of glomerulonephritis and fifteen subjects with miscellaneous diseases were determined, using ion exchange chromatography.

2. The seromuroid of normal subjects contained an average of 91 per cent glucosamine (86 to 97 per cent) and 9 per cent galactosamine (3 to 14 per cent), or a mean galactosamine to glucosamine ratio of 0.10.

3. Although the total seromuroid of the patients with connective tissue diseases was increased, the galactosamine to glucosamine ratio remained essentially the same as in the normal.

4. There was a modest decrease in total seromuroid and of seromuroid glucosamine in the nephrotic phase of glomerulonephritis, whereas there was an increase in the galactosamine resulting in a distinctly increased galactosamine to glucosamine ratio. This abnormality tended to return toward normal during therapy-induced remissions.

5. A slight increase in galactosamine to glucosamine ratio was noted in several of the sub-

jects in the miscellaneous group including two myxedematous patients, one with parathyroid adenoma, and one with chronic glomerulonephritis. The remainder of this heterogeneous group had normal ratios of the two hexosamines.

6. It was shown that, whereas galactosamine was readily demonstrable in whole serum hydrolysates, the two mucopolysaccharide fractions prepared by mild alkaline treatment of serum and containing 57 and 8 per cent of the total serum hexosamine contained only glucosamine. This was true in both the normal and nephrotic.

7. The implications of these findings upon sero-mucoid metabolism in these diseases are discussed.

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