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Research Article

CONTENT AND COMPOSITION OF THE MUCOPROTEIN FRACTION OF HUMAN SERUM (SEROMUCOID) IN DISEASE, WITH SPECIAL REFERENCE TO HEMATOLOGIC DISORDERS [1]

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Significant alterations of content, distribution, electrophoretic mobility and, possibly, composition of carbohydrate-containing proteins in many diseases have been described by both chemical and electrophoretic methods. From a summary of the pertinent observations in the literature (Table I), it may be concluded that the carbohydrate content of proteins is high in many disease states; also, that the electrophoretic distribution of polysaccharides or their chemical precipitation with the various protein fractions may often be abnormal. Thus, the study of carbohydrate-containing proteins of serum is a potentially important and significant tool in the detection of basic molecular alterations, which may be significant in the understanding of the pathogenesis of disease.

We have attempted in a number of disease states a systematic study of the content and in the chemical composition of one type of carbohydrate-containing proteins, the mucoproteins. Because of the major interest of our Institution and our previous work on the subject particular emphasis was directed to hematologic disorders. The serum mucoprotein fraction was selected for this study because: a) it shows significant and wide variations in disease (Table I); b) fairly reliable techniques are available for its isolation and for the analysis of some of its constituents. Total mucoproteins, mucoprotein-bound hexoses and hexosamine were studied. For comparison, values for total protein, total hexosamine and total non-glucosamine protein-bound hexoses were also obtained for all sera. Our work was handicapped by the present confusion in classification and definition of the polysaccharide-containing proteins. These have been divided by Meyer (1) into glycoproteins and mucoproteins. The two types appear similarly constituted with a mucopolysaccharide held to a protein moiety by a firm chemical bond. However, in mucoproteins, the degree of alcohol solubility is higher and the hexosamine content is more than 4 per cent weight. In glycoproteins, hexosamine represents less than 4 per cent. In this study the definition of mucoprotein, as suggested by Winzler, Devor, Mehl, and Smyth (2), was applied to that fraction of serum proteins soluble in perchloric acid solution, and precipitable by phosphotungstic acid. The fraction we studied appeared comparable to the seromucoid of Rimington (3) and the seroglycoid of Hewitt (4). Since we considered as mucoproteins a group of proteins of serum on criteria of solubility alone, only relative conclusions were possible. In particular, our findings have no bearing on the exact nature of mucoproteins and on their relationship to other carbohydrate-containing proteins.

METHODS

Total serum protein (T.P.) was determined by the method of Gornall, Bardawill, and David (5), which employs the biuret reaction; total non-glucosamine, protein-bound hexoses (T.Ph.) by the procedure of Sheppard and Everett (6); total hexosamine (T.Ha) by the method of Rimington (3); (d) serum mucoproteins (T.Mp.) were separated as described by Winzler and co-workers (2). This method is based on the precipita-

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TABLE I

Serum total hexoses, protein-bound hexoses, total hexosamine and mucoprotein in disease: A summary *

Total hexoses	*Elevated* in advanced tuberculosis (21, 22, 16), sarcoidosis and cancer (22, 16), rheumatic fever in relapse (9), rheumatoid arthritis (15), diabetes mellitus with complications (14), disseminated malignancies (23, 24), leukemia, lymphoma, chronic infections, colitis, acute glomerulonephritis, prostatic hypertrophy (24).
Protein-bound hexoses	*Elevated* in carcinoma, benign tumors, arthritis, bacterial infections (17), disseminated malignancies (25, 26), tuberculosis (27, 28). *Hexoses in serum albumin fraction:* elevated in normal pregnancy (29), more so in carcinoma (17) and rheumatic fever (9). In multiple myeloma, the pathological protein contains maximum amount of polysaccharides (30). In pneumonia, polysaccharides are high in all electrophoretic fractions (31).
Protein-bound hexoses: total protein ratio	*Elevated* in rheumatoid arthritis, in relation to severity of symptoms (32).
Total hexosamine	*Elevated* in rheumatic fever (9), pneumonia (33), carcinoma. Hodgkin's disease, leukemias, rheumatoid arthritis, periarteritis nodosa, rheumatic fever, chronic infection, pneumonia, myocardial infarction (34), lupus erythematosus (35), gout, cirrhosis of the liver, hepatitis (18). Hexosamine is highest in the gamma globulin fraction of patients with lupus erythematosus (36). In other diseases, the α_1-globulin contains most of the increased hexosamine (18).
Mucoprotein (seromucoid)	*Elevated* in diabetes mellitus with complications (14), myocardial infarction (37), carcinoma and pneumonia (19), rheumatic fever (38), trauma (12). *Decreased* in liver disease, some endocrine dysfunctions, nephrosis (12).
Mucoprotein-bound hexoses	*Elevated* in rheumatoid arthritis (15).

* The terminology used in this table is in agreement with that used in the text.

tion of all proteins by perchloric acid and subsequent isolation of mucoproteins which are insoluble in phosphotungstic acid.[6] After isolation, the following components of mucoproteins were determined: a) protein moiety (T.Mp.); b) hexoses (Mh.); c) hexosamine (Mha.). Mean values and standard deviation were calculated for each value according to the formula:

$$\sigma = \sqrt{\frac{\Sigma i(\bar{x} - xi)^2}{n}},$$

where $\bar{x}$ represented the mean, xi the i^{th} individual determination, and n the number of determinations. The significance of our results was further evaluated by means of the formula (7):

$$t = \frac{\overline{X}_1 - \overline{X}_2}{S_{1-2}} \sqrt{\frac{N_1 \cdot N_2}{n_1 + n_2}}$$

where S_{1-2} equalled

$$\sqrt{\frac{\Sigma x_1^2 + \Sigma x_2^2}{N_1 + N_2 - 2}}.$$

In the latter formula, $\overline{X}_1$ indicated the mean of normal values; $\overline{X}_2$, the mean of the values being investigated; N_1, the number of samples in the normal group; N_2, the number of samples in the group investigated; x_1 the square root of the differences of each sample in the normal group from the mean value; x_2 the same value for the group investigated. The significance of t values was judged on the basis of a standard table (8).

Materials (Table IV)

Eight healthy individuals and 111 patients were included in this study. Eighty-eight patients represented cases seen in the wards of our Institution. Serum for an additional twenty-three patients was obtained through the courtesy of Drs. Jean Dausset (Centre National des Transfusions Sanguines, Paris, France), James Monto (Henry Ford Hospital, Detroit, Michigan) and Donat Cyr (Lahey Clinic, Boston, Massachusetts). These samples may be recognized in all tables since they are indicated by number, rather than by patient's initials; they were included in the series to verify the reliability of results against possible errors in diagnosis. Serum from local patients was promptly frozen and stored at $-20°$ C. until used. Sera from other areas were sent by air in containers filled with dry ice. In each in-

[6] Winzler and co-workers (2) have indicated that dilution of serum and final molarity of perchloric acid are essential to optimal precipitation of a constant amount of mucoprotein. They have advised a concentration of 0.6 M perchloric acid. Results indicated in Tables II and III show that dilution and molarity of perchloric acid were of relatively little bearing on the final yield of mucoproteins. Thus, a molarity of 0.24 M was used in the course of this study, a figure which seemed to assure the complete precipitation of the total protein fraction with a minimum amount of reagent. The serum was diluted 1:5 with perchloric reagent as suggested by Winzler.

TABLE II

Effect of the dilution of serum on the recovery of mucoproteins and their composition: A representative experiment

		Weight of dry precipitate	Composition of mucoproteins		
			Protein	Hexoses	Hexosamine
		mg. %	*mg. %*	*mg. %*	*mg. %*
(a) Normal serum dilution	1:1	83	80	4.2	9.6
	1:2	83	80.9	4.21	9.7
	1:5	82	82	4.29	9.77
(b) Abnormal serum dilution (lymphosarcoma: S. R., ♀, 38)	1:1	296	296	34	15.5
	1:2	295.3	295.5	33.4	15.25
	1:5	296	296	33.6	15.4

TABLE III

Total mucoprotein (T.Mp.), mucoprotein-bound hexoses (Mh.) and hexosamine (Mha.) from serum treated with decreasing concentration of perchloric acid *

Molarities of perchloric acid	0.6			0.45			0.3			0.225			0.15		
	T.Mp.	Mh.	Mha.	T.Mp.	Mh.	Mha.	T.Mp.	Mh.	Mha.	T.Mp.	Mh.	Mha.	T.Mp.	Mh.	Mha.
	mg. %	*mg. %*	*mg. %*	*mg. %*	*mg. %*	*mg. %*	*mg. %*	*mg. %*	*mg. %*	*mg. %*	*mg. %*	*mg. %*	*mg. %*	*mg. %*	*mg. %*
Normal serum	98.8	6.2	11.5	98.6	6.2	11.5	98.8	6.1	11.6	98.8	6.1	11.5	98	5.75	11
	100	6.3	11.7	101	5.2	11.7	100	6.1	11.5	98	6.2	11.6	92	5.6	10.9
Pathological serum	190	12.6	15.1	190	12.5	15	190	12.6	15	189	12.6	15	162	12	14.5
	218	13.0	19.1	217	12.9	19.0	218	12.9	19.1	218	13.0	19.2	209	11.9	17.3

* Averages of duplicate experiments on each serum.

stance, diagnosis was established by the usual hematologic criteria, and proven by either biopsy of involved tissues or autopsy findings. Since many diseases are accompanied by major alterations in content of serum glycoproteins and mucoproteins, cases selected for the present study, as far as could be determined, were free from infections, thrombosis, or hepatic and renal disorders. No definitive data appeared available to indicate whether treatment (myelo-suppressive drugs, X-ray radiation, and so on) may influence the level and composition of mucoproteins.[7] In our experience, the amount and the composition of the serum mucoprotein fraction were more closely related to the state of remission or relapse, than to the effects of therapy. Usually, therefore, blood was collected from patients in clinical relapse, who were untreated at the time of blood collection.

RESULTS

The conclusions to follow are based primarily on the results of the t test as a criterion of the statistical significance of our findings.

[7] Steroids, chemotherapeutic agents of various nature, X-ray radiation, and so on might considerably modify the content and composition of mucoproteins in disease. The elevated content of mucopolysaccharide in the serum of patients with rheumatic fever decreases significantly following steroid therapy (9). In guinea pigs (10) and in rats (11), however, no correlation has been found between administration of steroids and variations in protein-bound hexoses.

A. Normal sera (Table V)

Our findings agreed closely with those reported in the literature. They represented base values with which abnormal findings were compared.

TABLE IV

Sera studied in the course of these observations

Iron deficiency anemia		4	
Pernicious anemia		6	
Polycythemia vera		4	
Myeloid metaplasia		4	
Congenital spherocytosis		4	
Acute leukemia:	myelogenous	5	
	histiocytic	3	11
	erythroblastic	1	
	unclassified	2	
Chronic leukemia:	myelogenous	7	16
	lymphocytic	9	
Hodgkin's disease:	granuloma	5	10
	sarcoma	5	
Lymphoblastoma		3	
Lymphosarcoma		9	
Reticulum cell sarcoma		8	
Mycosis fungoides		5	
Multiple myeloma		6	
Acquired auto-immune hemolytic anemia		3	
Idiopathic thrombocytopenic purpura		5	
Lupus erythematosis disseminatus		5	
Disseminated malignancies		8	
			111
Normal sera		8	
Total			119

TABLE V

Serum protein-bound hexoses in normal subjects *

Subject	T.P.	T.Ph.	T.Ha.	T.Mp.	Mh.	Mha.
	mg. %	*mg. %*	*mg. %*	*mg. %*	*mg. %*	*mg. %*
S. M., ♂, 28	7,600	100	100	95	5.355	10.20
M. M., ♀, 28	6,800	95	85	90	4.165	10.20
E. M., ♀, 29	7,000	125	107	105	8.500	12.75
W. B., ♂, 24	7,000	99	88	115	10.200	12.75
R. M., ♀, 32	7,200	120	100	85	4.250	9.35
N. M., ♀, 25	7,700	115	105	100	7.140	11.90
M. S., ♂, 38	7,500	107	98	90	4.505	9.35
S. K., ♂, 29	7,900	115	106	110	7.650	13.60
Average	7,337.5	109.5	98.6	98.8	6.471	11.26
σ	367.21	10.17	7.647	9.92	2.098	1.58

* As in each table, analytical values and standard deviation (σ) are given for the following: Total protein (T.P.), protein-bound hexoses (T.Ph.), total hexosamine (T.Ha), total mucoprotein (T.Mp.), mucoprotein-bound hexoses (Mh.), and mucoprotein-bound hexosamine (Mha).

TABLE VI

Serum protein-bound hexoses in acute leukemia

Type	Case	T.P.	T.Ph.	T.Ha.	T.Mp.	Mh.	Mha.
		mg. %	*mg. %*	*mg. %*	*mg. %*	*mg. %*	*mg. %*
Unclassified	No. 3005	7,300	138	128	186	8.84	13.60
Granulocytic	No. 11	7,600	130	115	246	27.63	23.80
Granulocytic	No. 12	7,800	135	120	171	24.40	21.25
Granulocytic	No. 13	7,600	160	150	228	12.50	12.75
Histiocytic	D. M., ♀, 38	7,200	147	140	310	42.50	12.75
Granulocytic	L. S., ♀, 76	7,200	147	120	253	38.25	10.20
Histiocytic	C. P., ♂, 29	7,300	150	120	103	15.30	13.60
Erythroblastic	P. McG., ♂, 52	7,000	160	140	123	20.91	18.70
Lymphocytic	J. T., ♂, 5	6,100	190	120	123	8.67	10.20
Granulocytic	C. V., ♀, 54	7,000	190	140	140	16.58	15.30
Granulocytic	R. Q., ♂, 57	6,100	185	110	117	9.69	11.90
Average		7,109.1	157.5	127.5	181.8	20.48	14.91
σ		531.64	19.88	12.3	65.34	11.14	4.26
t		0.989	5.63	4.95	3.28	4.38	2.15

TABLE VII

Serum protein-bound hexoses in chronic leukemia (myelogenous and lymphocytic)

Case	Type	T.P.	T.Ph.	T.Ha.	T.Mp.	Mh.	Mha.
		mg. %	*mg. %*	*mg. %*	*mg. %*	*mg. %*	*mg. %*
G. D., ♀, 53	Granulocytic	5,900	125	110	113	12.50	13.60
J. O., ♂, 29	Granulocytic	6,900	130	115	118.6	15.30	17.00
No. 7	Granulocytic	6,000	85	120	105.2	15.56	14.45
No. 8	Granulocytic	6,000	175	145	155	9.78	12.75
No. 9	Granulocytic	6,500	135	118	165	10.29	11.05
E. S., ♀, 70	Granulocytic	7,300	152	120	145	9.35	10.20
L. H., ♀, 47	Granulocytic	7,000	130	110	130	8.16	11.90
No. 102	Lymphocytic	7,600	125	115	128	17.00	18.70
No. 103	Lymphocytic	7,200	140	150	140	13.69	15.30
No. 104	Lymphocytic	6,300	135	130	140	18.28	17.00
No. 105	Lymphocytic	5,800	145	170	228	32.90	16.15
No. 2803	Lymphocytic	7,700	130	105	100	11.22	10.20
C. A., ♂, 70	Lymphocytic	6,400	135	95	92	11.05	8.50
J. P., ♂, 52	Lymphocytic	5,800	115	128	100	10.20	11.90
J. Q., ♂, 57	Lymphocytic	7,400	140	150	134	10.12	11.05
J. B., ♂, 48	Lymphocytic	6,700	134	160	195	15.30	14.45
Average		6,656.3	133.2	127.6	136.8	13.79	13.39
σ		639.31	17.96	20.73	35.05	5.73	2.827
t		2.67	3.26	3.68	2.8	3.2	1.88

TABLE VIII

Serum protein-bound hexoses in Hodgkin's disease

Case	Type	T.P.	T.Ph.	T.Ha.	T.Mp.	Mh.	Mha.
		mg. %	*mg. %*	*mg. %*	*mg. %*	*mg. %*	*mg. %*
M. d'A., ♂, 9	Sarcoma	6,700	230	170	190	32.30	34.00
M. F., ♀, 61	Sarcoma	6,200	158	95	158	9.18	15.30
G. M., ♂, 42	Sarcoma	6,500	190	98	100	8.67	14.45
E. G., ♂, 35	Sarcoma	7,700	200	175	204	7.74	13.60
A. M., ♀, 38	Sarcoma	6,700	200	145	210	22.95	15.30
I. diR., ♀, 19	Sarcoma	7,300	221	155	190	15.73	9.35
No. 2811	Granuloma	7,800	92	100	103	11.31	12.75
No. 2930	Granuloma	7,000	215	140	213	11.90	12.75
No. 71	Granuloma	6,800	198	160	175	13.60	11.90
C. M., ♀, 29	Granuloma	6,500	225	182	230	26.35	16.15
A. F., ♀, 61	Granuloma	7,600	153	130	153	5.19	12.75
Average		6,981.8	189.3	141	175	14.99	15.3
σ		516.67	38.92	30.18	43.66	8.201	6.183
t		1.76	5.48	3.65	4.81	2.73	1.72

B. Acute leukemia (Table VI)

There was a statistically significant rise of total hexosamine, total mucoprotein and hexoses bound to protein and mucoprotein fractions.

C. Chronic leukemias (Table VII)

The findings, essentially independent of the histologic type of the disease, were similar to those observed in the acute leukemias, although quantitatively less significant.

D. Hodgkin's disease (Table VIII)

There was a substantial rise in total hexosamine, total mucoprotein and protein-bound hexoses. The rise in mucoprotein-bound hexoses seemed statistically less significant. No appreciable difference was observed between the granuloma and the sarcoma type of the disease.

E. Lymphosarcoma (Table IX)

Total hexosamine, total mucoprotein and mucoprotein-bound hexoses were significantly increased. There was, in fact, a rather spectacular rise in total mucoprotein and mucoprotein-bound hexoses, which seemed typical of the disease. There was no significant difference in findings between patients in this group who had been classified by the pathologist as suffering from either lymphosarcoma or lymphoblastoma.

TABLE IX

Serum protein-bound hexoses in lymphosarcoma

Case	T.P.	T.Ph.	T.Ha.	T.Mp.	Mh.	Mha.
	mg. %	*mg. %*	*mg. %*	*mg. %*	*mg. %*	*mg. %*
R. Z., ♂, 57	6,100	207	150	211	34.27	11.90
No. 36	5,000	190	140	190	51.00	17.85
No. 37	7,500	203	180	301.2	46.75	18.70
No. 38	6,100	135	100	115	10.88	9.35
T. B., ♀, 50	7,700	190	158	200	32.05	11.90
E. M., ♀, 34	7,200	230	170	239	38.93	13.60
S. R., ♀, 38	7,800	255	160	295	33.92	15.30
H. C., ♂, 72	7,000	218	125	179	19.13	11.90
No. 39	7,200	203	120	195	38.68	11.90
T. B., ♀, 54	5,800	290	120	197	29.75	11.90
S. T., ♂, 78	5,600	240	170	279	34.00	13.60
C. M., ♀, 84	7,600	195	140	242	42.50	13.60
E. C., ♂, 43	6,300	280	120	181	31.88	14.45
Average	6,684.6	218.2	142.5	217.2	34.13	13.53
σ	878.67	32.74	23.48	50.42	10.19	2.48
t	1.97	2.33	4.9	6.16	7.07	2.19

TABLE X

Serum protein-bound hexoses in reticulum-cell sarcoma

Case	T.P.	T.Ph.	T.Ha.	T.Mp.	Mh.	Mha.
	mg. %	*mg. %*	*mg. %*	*mg. %*	*mg. %*	*mg. %*
C. B., ♀, 57	6,800	245	170	394.0	38.25	13.60
W. B., ♂, 80	5,600	205	140	160.7	7.74	13.60
J. M., ♂, 51	6,000	210	165	217.0	7.40	16.15
No. 34	7,600	275	130	153.0	32.47	12.75
M. C., ♂, 67	6,500	180	110	195.0	10.80	11.05
Average	6,500	223	143	223.9	19.33	13.43
σ	687.02	33.24	22.27	88.18	13.27	1.648
t	2.62	8.17	4.7	3.62	2.51	2.18

TABLE XI

Serum protein-bound hexoses in mycosis fungoides

Case	T.P.	T.Ph.	T.Ha.	T.Mp.	Mh.	Mha.
	mg. %	*mg. %*	*mg. %*	*mg. %*	*mg. %*	*mg. %*
T. C.	7,600	140	125	273	25.245	11.05
T. H.	7,200	135	110	183	21.505	8.50
P. C.	4,400	135	120	127	24.820	9.35
S. A.	5,600	130	160	137	16.745	6.80
B. B.	6,300	139	204	180	22.270	10.20
Average	6,220	135.8	143.8	180	22.117	9.18
σ	1,146.12	3.54	34.46	51.62	3.044	1.462
t	2.34	4.74	3.28	3.97	9.46	2.2

TABLE XII

Serum protein-bound hexoses in lupus erythematosus disseminatus

Case	T.P.	T.Ph.	T.Ha.	T.Mp.	Mh.	Mha.
	mg. %	*mg. %*	*mg. %*	*mg. %*	*mg. %*	*mg. %*
E. M., ♂, 78	7,200	120	150	195.0	23.375	11.90
H. McC., ♀, 33	6,200	107	135	68.9	6.96	11.05
No. 51	7,400	150	90	190.0	34.00	13.60
Sr. E., ♀, 54	7,600	140	140	123.0	9.18	11.05
S. O'H., ♀, 73	7,100	168.4	155.0	166.0	11.64	12.75
Average	7,100	137.1	134	148.6	17.03	12.07
σ	481.66	21.69	23.108	46.91	10.201	0.9913
t	0.92	2.77	3.68	2.53	2.68	0.93

TABLE XIII

Serum protein-bound hexoses in multiple myeloma

Case	Type	T.P.	T.Ph.	T.Ha.	T.Mp.	Mh.	Mha.
		mg. %	*mg. %*	*mg. %*	*mg. %*	*mg. %*	*mg. %*
G. G., ♂, 57	Plasmocytic leukemia	10,000	400	170	91	3.57	18.70
No. 21	β-myeloma	9,500	200	160	217	22.27	20.40
N. B., ♀, 62	β-myeloma	10,800	300	180	242	11.15	16.15
J. C., ♂, 59	γ-myeloma	9,100	180	160	117	11.05	17.00
D. S., ♂, 72	γ-myeloma	10,200	200	150	131	19.55	17.00
No. 22	γ-myeloma	9,000	250	170	190	17.00	15.30
Average		9,766.6	255	165	165	14.1	17.43
σ		634.21	76.103	9.574	55.06	6.242	1.68
t		7.6	4.51	12.16	2.81	2.72	5.95

TABLE XIV

Serum protein-bound hexoses in idiopathic thrombocytopenic purpura

Case	Type	T.P.	T.Ph.	T.Ha.	T.Mp.	Mh.	Mha.
		mg. %	*mg. %*	*mg. %*	*mg. %*	*mg. %*	*mg. %*
D. H., ♀, 5	Acute	7,600	180	125	92	10.6	12
K. K., ♀, 32	Chronic	6,500	185	160	100	12	15
M. C., ♀, 57	Acute	7,800	218	150	140	11.2	14
I. G., ♀, 56	Chronic	7,200	145	130	125	9.6	16
M. R., ♀, 28	Chronic	7,500	175	150	145	8.9	15
Average		7,320	180.6	143	120.4	10.46	14.4
σ		453.43	23.31	13.27	21.13	1.105	1.357
t		0.07	6.79	6.83	2.29	3.61	3.39

F. Reticulum-cell sarcoma (Table X)

Patients included in this group showed considerable variations in histologic characteristics and clinical course, but equal variability of chemical findings. The statistical analysis revealed significant rise in total hexosamine and protein-bound hexoses only.

G. Mycosis fungoides (Table XI)

There was a significant rise in hexoses bound to both the protein and the mucoprotein fraction, although the increase in total mucoprotein was equivocal. The rise in total hexosamine indicated by the value of σ was apparently of no real significance according to the evaluation of the t test.

H. Lupus erythematosus (Table XII)

There was a superficial resemblance of the findings to those in mycosis fungoides. Statistically, however, the only significant variation was an equivocal rise of the total hexosamine.

I. Multiple myeloma (Table XIII)

Serum of these patients showed a typical pattern. There was a significant rise in total protein, protein-bound hexoses, total and mucoprotein-bound hexosamine.

J. Idiopathic thrombocytopenic purpura (Table XIV)

Total hexosamine and protein-bound hexoses were significantly elevated. The rise in total mucoprotein was equivocal.

K. Disseminated malignancies (Table XV)

Although the sera studied in this group were obtained from a heterogenous group of patients with regard to site of the primary lesion, findings were uniform. There was significant decrease

TABLE XV

Serum protein-bound hexoses in generalized malignancies

Case	Type	T.P.	T.Ph.	T.Ha.	T.Mp.	Mh.	Mha.
		mg. %	*mg. %*	*mg. %*	*mg. %*	*mg. %*	*mg. %*
R. R., ♂, 62	Melanosarcoma	6,000	180	130	210.6	11.5	14.0
R. P., ♀, 52	Adenoacanthoma of ovary	6,900	190	135	215	10.2	13.0
H. H., ♀, 60	Adenocarcinoma of breast	6,300	185	130	220	12.5	14.5
J. N., ♂, 60	Carcinoma of prostate	5,900	170	125	177	11.3	13.2
H. H., ♂, 36	Anaplastic carcinoma	5,200	175	138	196	12.2	14.4
H. N., ♂, 55	Carcinoma of rectum	6,700	164	125	173	9.1	13.0
L. S., ♀, 38	Carcinoma of stomach	4,500	167	130	180	10.0	13.8
N. H., ♀, 67	Carcinoma of stomach	7,100	185	142	209	12.7	14.6
Average		6,075	177	131.9	197.6	11.2	13.8
σ		825.76	8.86	5.644	17.48	1.219	0.629
t		4.98	11.94	9.38	14.31	6.84	3.92

in total proteins, with significant elevation of all other serum constituents. Total hexosamine seemed relatively more increased than hexosamine bound to the mucoprotein fraction.

L. Miscellaneous group

Our findings were normal in 25 patients with such conditions as polycythemia vera (four), acquired idiopathic auto-immune hemolytic anemia without evidence of collagen disease or malignancy (three), congenital spherocytosis (four), pernicious anemia (six), iron deficiency anemia (four) and myeloid metaplasia (four).

DISCUSSION

The significance of quantitative changes of the serum mucoprotein fraction in disease has been fully reviewed by Greenspan (12). This author concluded that the total mucoprotein fraction of serum is increased in trauma and in diseases accompanied by inflammatory, neoplastic or degenerative tissue changes, while it is decreased in parenchymal hepatic disease, endocrine dysfunctions and nephrosis. Only fragmentary work, however, seems to have been done on the qualitative composition of the serum mucoprotein fraction in disease. This paper attempts to correlate changes in the mucoprotein fraction of serum in patients with several hematologic diseases as compared with normal sera. This has been done through the study of quantitative variations of some constituents of the serum mucoprotein fraction and of their comparative distribution between the protein and mucoprotein fractions.

Figure 1 and Table XVI are a summary of our work. In Figure 1, the height of columns is based on the calculated averages. With few exceptions, these variations appear significant, as indicated by the results of the t test whose evaluation is offered in Table XVI and is the base for the discussion to follow. The decrease of total protein, noted in most conditions studied on the basis of average figures, was significant only in disseminated malignancies. Absolute rise of serum mucoprotein seemed a common denominator of most conditions studied although significant only in neoplastic processes (acute leukemias, Hodgkin's disease, lymphosarcoma and disseminated malignancies). An increase in protein-bound hexoses was again noted in practically all conditions studied and was statistically significant in the majority of them (acute and chronic leukemias, Hodgkin's disease, reticulum cell sarcomas, mycosis fungoides, multiple myeloma, idiopathic thrombocytopenic purpura and disseminated malignancies). In some conditions (acute and chronic leukemias, mycosis fungoides, idiopathic thrombocytopenic purpura, disseminated malignancies) there was significant increase in mucoprotein-bound hexoses while interesting dissociations were also observed. Thus in Hodgkin's disease and in multiple myeloma only the rise in protein-bound hexoses was significant; in lymphosarcoma, only the rise of mucoprotein-bound hexoses. In mycosis fungoides the rise in mucoprotein-bound hexoses was much more significant than that of the hexoses bound to protein. The opposite situation held true for idiopathic thrombocytopenic purpura. Total hexosamine was also significantly increased in acute and chronic leukemias, Hodgkin's disease, lymphosarcoma, reticulum cell sarcoma, multiple myeloma, idiopathic thrombocytopenic purpura

TABLE XVI

Statistical evaluation of results by the t test: A summary *

	T.P.	T.Ph.	T.Ha.	T.Mp.	Mh.	Mha.
Acute leukemias	−	+	+	+	+	−
Chronic leukemias	±	+	+	±	+	−
Hodgkin's disease	−	+	+	+	−	−
Lymphosarcoma	−	−	+	+	++	−
Reticulum cell sarcoma	−	+	+	−	−	−
Mycosis fungoides	−	+	−	±	++	−
Lupus erythematosus	−	−	±	−	−	−
Multiple myeloma	+	+	++	−	−	+
Idiopathic thrombocytopenic purpura	−	+	+	−	±	−
Generalized malignancies	+(↓)	++	++	++	+	+

* −, insignificant; ±, equivocal; +, significant; and ++, very significant. Variations represented increase from normal with the exception of the group "generalized malignancies," where values for T.P. were lower than normal.

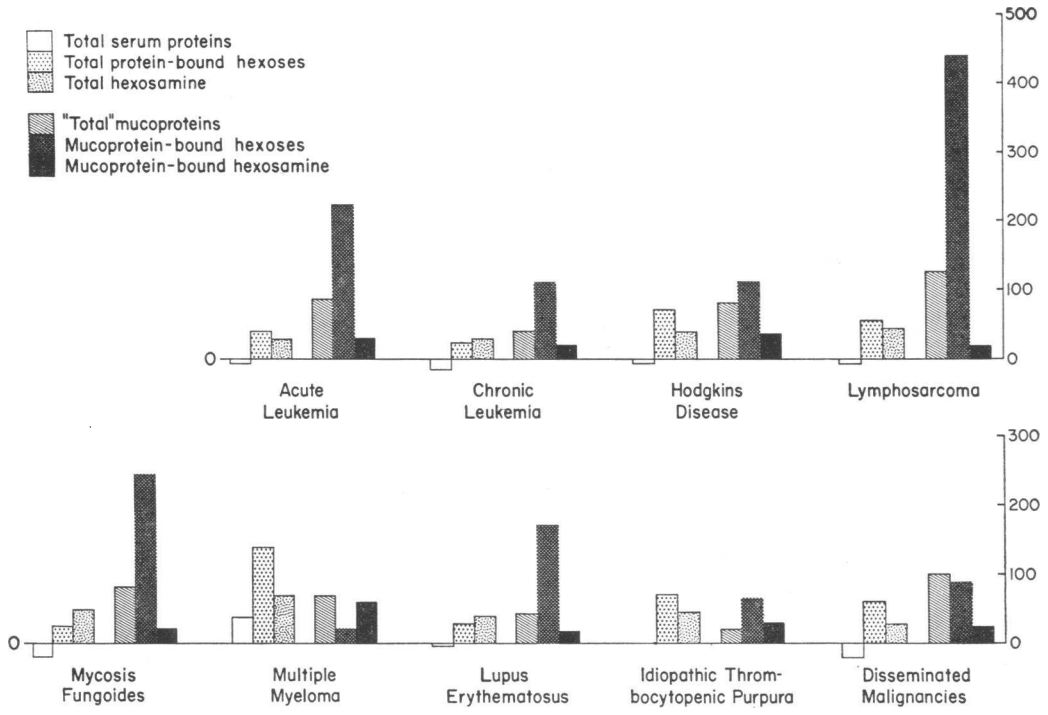

FIG. 1. COMPOSITION OF MUCOPROTEINS IN VARIOUS HEMATOLOGIC DISORDERS—A SUMMARY

Figures represent percentage variations from average normal values, indicated by the horizontal line. Also given for comparison are variations in serum total proteins, total protein-bound hexoses and total hexosamine.

and disseminated malignancies; rise was equivocal in lupus erythematosus. It was of interest that mucoprotein-bound hexosamine was significantly elevated only in multiple myeloma and in disseminated malignancies. In a few instances, the combined variations of the serum constituents produced a distinctive pattern, as in multiple myeloma, lymphosarcoma, and disseminated malignancies. The potential diagnostic value of the changes observed, however, should perhaps be minimized, as these occurred only in patients in fairly advanced stages of the disease.

Also of interest were the findings indicating that the distribution of hexoses between the protein and mucoprotein fractions and the ratio of total hexosamine to mucoprotein-bound hexosamine were much different in normal than in many pathologic conditions. These findings raised the necessary question as to whether the composition of the serum mucoprotein fraction may be different in health as compared to disease and, perhaps, even from one disease state to another. Unpublished electrophoretic studies in this Laboratory have given further support to this possibility, showing an overall anomaly of electrophoretic migration of mucoprotein in disease. Thus, its constituents are usually recovered from the α_1-, α_2-, and β-fractions; in pathologic conditions they may be recovered from the γ-fraction. Other supportive evidence can be obtained from the literature which suggests the largest amount of protein-bound hexoses to be found with the α-globulins in most disease states (13–15). In carcinoma and in rheumatic fever, however, protein-bound hexoses are most abundant in the albumin fraction (16, 17). Bollett (18) has recently confirmed an increase of hexosamine in the mucoprotein fraction of serum in a variety of illnesses, obtaining results which are essentially similar to ours when the same disease states were studied. He found also that such increase was limited to the hexosamine recovered from the α_1 paper electrophoretic fraction. Finally, there is additional evidence, although fragmentary, of a chemical nature. An increase in the ratio hexose: tyrosine in the mucoprotein fraction has been reported in

rheumatoid arthritis, gout (15) and experimental scurvy; it is not found, however, in many other conditions where this ratio has been studied (19, 20).

All these data seem to support the hypothesis that the mucoprotein fraction of serum in disease may be of abnormal composition. It should be remembered, however, that this fraction is of considerable heterogeneity and is possibly nothing more than a mosaic, combining several units. A single increase in content of one of the various fractions might well result in a simulated abnormal structure for the entire mucoprotein.

SUMMARY

1. Total protein, total protein-bound hexoses, total hexosamine, total mucoprotein, mucoprotein-bound hexoses and hexosamine were studied in a series of 111 patients with various hematologic disorders and in 8 healthy individuals.

2. The mucoprotein fraction and total hexosamine were quantitatively increased in diseases of a neoplastic nature. Significant variations of the protein-bound and mucoprotein-bound hexoses were observed in many instances. Fairly distinctive patterns were found in a few diseases (multiple myeloma, lymphosarcoma, and disseminated malignancies).

3. The various anomalies observed suggested that the composition of the mucoprotein fraction of serum may be abnormal in disease.

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