SERUM PROTEIN-BOUND CARBOHYDRATES IN RHEUMATIC DISEASE

I. RESULTS OF DIFFERENTIATED ANALYSES IN VARIOUS RHEUMATIC DISORDERS

BY

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The differential diagnosis between rheumatoid arthritis (RA) with severe general symptoms and systemic lupus erythematosus (SLE) with marked articular involvement may be very difficult, as is sometimes the differentiation between other rheumatic disorders. The possibility of using the diphenylamine reaction (test for sialic acid) as an aid in differential diagnosis has been discussed by Adam, Malecek, and Kútová (1957). Stidworthy, Payne, Shetlar, and Shetlar (1957) recommended the determination of protein-bound hexoses as a sign of activity and Bollet (1957) analysed protein-bound hexosamines, but we know no series wherein all these components have been analysed by chemical methods. We have studied a group of nearly 200 patients with rheumatic diseases to find out whether detailed analyses of serum protein-bound carbohydrates were of value in differential diagnosis. The protein-bound carbohydrates (hexoses, hexosamines, and sialic acid) were analysed in whole serum and after zone electrophoretic separation in polyvinyl chloride.

Material

The series comprised 187 in- and out-patients from the Department of Rheumatology, Karolinska Sjukhuset. The diagnostic criteria used were as follows:

Rheumatoid Arthritis (RA).—All 61 patients in this group fulfilled the diagnostic criteria of Ropes, Bennett, Cobb, Jacox, and Jessar (1959). The rheumatoid factor test was positive in 53 of them.

Systemic Lupus Erythematosus (SLE).—Ten patients had arthralgia or arthritis, signs of visceral involvement, increased serum gamma globulin levels, and a positive L.E.-cell test at some time or other. Only one patient in this group had a positive haemagglutination test for rheumatoid factor.

Arthritis and Arthralgia Secondary to Infection.—Of 56 patients in this group, fifteen had an acute arthritis and a focus of active infection at the time of the examination. In the other 41 cases there was a history of definite arthritis presenting after an acute bacterial infection. In most of them the erythrocyte sedimentation rate was still elevated. Tests for rheumatoid factor and L.E.-cells were always negative. No cases were seen of rheumatic fever with evidence of endocarditis. Cases of uro-arthritis were placed in a separate group (Kellgren, 1962).

Uro-arthritis.—This group of ten patients was diagnosed according to Olhagen (1960); it includes cases of post-gonorrhoeal arthritis and Reiter's syndrome.

Ankylosing Spondylitis.—Only the twelve cases in which x-ray examination showed bilateral sacro-iliac changes and paravertebral syndesmophytes characteristic of ankylosing spondylitis (Romanus, 1953) were included in this group.

Osteo-arthritis.—The 38 patients in this group had clinical and radiologically verified arthrotic lesions without signs of active arthritis. The erythrocyte sedimentation rate was normal in 23 and slightly raised in fifteen.

The composition of the series with regard to sex, age, and diagnosis is shown in Table I (overleaf).

Methods

Protein-bound Carbohydrates were determined by the following methods (Böttiger and Carlson, 1960a): Hexoses by the anthrone reagent.

Hexosamines by the Elson-Morgan procedure as modified by Blix.

Sialic acid by Svennerholm's resorcinol-Cu-reagent.

Total Protein.—A biuret method.

Erythrocyte Sedimentation Rate (ESR).—Westergren method.

Paper Electrophoresis.—In barbital buffer of pH 8·6 and ionic strength 0·1.

TABLE I
COMPOSITION OF THE SERIES

Diagnosis		No. of Patients			Mean Age (yrs)
		Men	Women	Total	
Rheumatoid Arthritis	Low activity ..	9	23	32	52
	High activity ..	5	24	29	
	Total ..	14	47	61	
Systemic Lupus Erythematosus	..	3	7	10	43
Postinfective Arthritis	Active ..	7	8	15	40
	Late ..	10	31	41	
	Total ..	17	39	56	
Uro-arthritis	..	8	2	10	37
Ankylosing Spondylitis	..	11	1	12	46
Osteo-arthritis	E.S.R. $\leq$ 20 mm./hr	8	15	23	55
	E.S.R. $\geq$ 21 mm./hr	3	12	15	
	Total ..	11	27	38	
	No. ..	64	123	187	
Total	Per cent.	34	66	100	

Zone Electrophoresis.—In polyvinyl chloride (Böttiger and Carlson, 1960b).

Results

The results of the whole serum analyses are presented in Table II, where the RA cases are shown in two groups, with low and high disease activity. The evaluation of activity in RA is to be discussed in a following paper (Böttiger, Malmquist, and Olhagen, 1964). Mean total protein-bound carbohydrates in the various groups are given in Fig. 1 (opposite), and the same mean values are plotted against the erythrocyte sedimentation rate in Fig. 2 (opposite).

The results of typical zone electrophoretic separations of RA and SLE sera are shown in Figs 3 and 4 (overleaf).

The mean values of four such separations of sera from RA patients and of three from SLE patients are given in Table III (overleaf).

The mean percentage carbohydrate calculated on protein content is given in Table IV (overleaf).

Discussion

The mean age is the same in the groups of RA and osteo-arthritis (52 and 55 years, respectively) and somewhat lower in the other groups. As no

TABLE II
RESULTS OF ANALYSES OF SERUM PROTEINS AND PROTEIN-BOUND CARBOHYDRATES
IN VARIOUS RHEUMATIC DISORDERS

(Mean values $\pm$ standard error of the mean and (below) standard deviation)

Disease		No. of Cases	Protein-bound Carbohydrates (mg./100 ml.)				Erythrocyte Sedimentation Rate (Westergren) (mm./hr)	Serum Proteins (g./100 ml.)		
			Hexoses	Hexo-samines	Sialic Acids	Total		Total	Alpha ₂ Globulin	Gamma Globulin
Rheumatoid Arthritis	Low activity ..	32	138 $\pm$ 4 24	112 $\pm$ 3 18	75 $\pm$ 2 14	328 $\pm$ 9 53	25 $\pm$ 3 19	7.25 $\pm$ 0.10 0.57	0.75 $\pm$ 0.03 0.14	1.60 $\pm$ 0.08 0.37
	High activity ..	29	153 $\pm$ 3 19	128 $\pm$ 3 16	88 $\pm$ 3 15	368 $\pm$ 8 43	43 $\pm$ 4 23	7.30 $\pm$ 0.04 0.50	0.88 $\pm$ 0.05 0.23	1.69 $\pm$ 0.06 0.28
Systemic Lupus Erythematosus	..	10	147 $\pm$ 10 31	121 $\pm$ 7 22	77 $\pm$ 7 22	345 $\pm$ 23 74	58 $\pm$ 11 36	7.45 $\pm$ 0.27 0.84	0.81 $\pm$ 0.10 0.29	2.36 $\pm$ 0.35 0.99
Post-infective Arthritis	Active ..	15	133 $\pm$ 7 26	110 $\pm$ 4 18	75 $\pm$ 3 12	318 $\pm$ 14 54	24 $\pm$ 5 21	7.41 $\pm$ 0.16 0.62	—	—
	Late ..	41	126 $\pm$ 2 16	105 $\pm$ 2 14	70 $\pm$ 2 11	301 $\pm$ 6 39	17 $\pm$ 2 14	7.37 $\pm$ 0.07 0.46	—	—
Uro-arthritis	..	10	147 $\pm$ 9 28	121 $\pm$ 7 23	86 $\pm$ 5 17	355 $\pm$ 20 66	29 $\pm$ 8 24	7.49 $\pm$ 0.13 0.42	0.86 $\pm$ 0.12 0.26	1.66 $\pm$ 0.10 0.19
Ankylosing Spondylitis	..	12	156 $\pm$ 10 33	126 $\pm$ 6 22	89 $\pm$ 5 17	371 $\pm$ 20 70	39 $\pm$ 7 24	7.41 $\pm$ 0.11 0.37	0.85 $\pm$ 0.06 0.17	1.57 $\pm$ 0.18 0.47
Osteo-arthritis	E.S.R. $\leq$ 20 mm./hr	23	116 $\pm$ 3 12	97 $\pm$ 2 11	61 $\pm$ 1 6	273 $\pm$ 5 26	9 $\pm$ 1 5	7.11 $\pm$ 0.01 0.42	—	—
	E.S.R. $\geq$ 21 mm./hr	15	126 $\pm$ 4 14	100 $\pm$ 2 7	66 $\pm$ 1 5	292 $\pm$ 6 22	24 $\pm$ 2 6	7.20 $\pm$ 0.01 0.33	—	—
Normal Subjects	..	257	112 $\pm$ 0.8 12.3	93 $\pm$ 0.6 9.5	67 $\pm$ 0.4 7.1	270 $\pm$ 1.6 25.0	—	7.29 $\pm$ 0.03 0.48	0.50 $\pm$ 0.02 —	1.08 $\pm$ 0.04 —

Fig. 1.—Mean total protein-bound carbohydrates in various rheumatic disorders. Normal range ($\bar{X} \pm 2$ S.D.) indicated by shaded area.

Fig. 2.—Mean total protein-bound carbohydrates plotted against erythrocyte sedimentation rates, with regression line for these values in rheumatoid arthritis with the exclusion of four patients with very high erythrocyte sedimentation rates and high gamma-globulin levels. Same groups as Fig. 1.

Fig. 3.—Zone electrophoretic separation of serum from a patient with active rheumatoid arthritis.

Fig. 4.—Zone electrophoretic separation of serum from a patient with active systemic lupus erythematosus.

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TABLE III

MEAN SERUM PROTEINS (g./100 ml.) AND TOTAL PROTEIN-BOUND CARBOHYDRATES (mg./100 ml.) (HEXOSES + HEXOSAMINES + SIALIC ACIDS) AFTER ZONE ELECTROPHORETIC SEPARATIONS OF SERA FROM PATIENTS WITH RHEUMATOID ARTHRITIS AND SYSTEMIC LUPUS ERYTHEMATOSUS

Diagnosis	No. of Cases	Estimations	Albumin	Globulin				Total
				alpha-1	alpha-2	beta	gamma	
Rheumatoid Arthritis	4	Serum Proteins	3.18	0.72	1.23	0.70	1.82	7.65
		Total protein-bound carbohydrates	16	132	208	63	95	514
Systemic Lupus Erythematosus	3	Serum Proteins	2.65	0.58	0.92	0.69	2.82	7.66
		Total protein-bound carbohydrates	9	124	156	54	99	442
Normal Subjects	10	Serum Proteins	4.00	0.26	0.58	0.47	1.02	6.33
		Total protein-bound carbohydrates	12	54	90	56	62	274

TABLE IV

MEAN PERCENTAGE PROTEIN-BOUND CARBOHYDRATES (HEXOSES + HEXOSAMINES + SIALIC ACIDS) IN VARIOUS PROTEIN FRACTIONS AFTER ZONE ELECTROPHORETIC SEPARATION

Diagnosis	No. of Cases	Albumin	Globulin			
			alpha-1	alpha-2	beta	gamma
Rheumatoid Arthritis	4	0.4	19	17	9	5
Systemic Lupus Erythematosus ..	3	0.3	21	17	8	4
Normal Subjects	10	0.3	21	16	11	6

The same agreement between the values for rheumatoid arthritis, systemic lupus erythematosus, and normal subjects is seen also for the single components (*i.e.* hexoses, hexosamines, and sialic acids) taken separately.

age-dependent increase in serum protein-bound carbohydrates has been found (Böttiger and Carlson, 1960; Böttiger and Holmström, 1964), these differences will not influence the results.

Excluding the cases of osteo-arthritis, it may be seen from Table II and Fig. 1 that all the rest show a definite increase in the values of all protein-bound carbohydrates analysed. No differences were found between the various components, the molecular ratios between hexoses, hexosamines, and sialic acids being the same in all the groups and also the same as in normal subjects (see discussion in Böttiger, Estborn, and Möllerberg, 1960), the highest values being found in RA, SLE, and ankylosing spondylitis. Fig. 2 shows a close correlation between the total protein-bound carbohydrates and the erythrocyte sedimentation rate in all groups, except SLE and perhaps osteo-arthritis. This deviating pattern in SLE is probably explained by the high gamma-globulin levels in this condition, which contribute to the increase in the sedimentation rate but having a low carbohydrate content do not increase the protein-bound carbohydrate. The difference in mean values for gamma-globulins in RA (1.65

g./100 ml.) and SLE (2.36 g./100 ml.) is highly significant ($P < 0.001$).

No electrophoretic analyses were made in the cases of osteo-arthritis. Patients with a normal ESR (mean 9 mm./hr) have entirely normal values for protein-bound carbohydrates. Those with a slightly elevated ESR (mean 24 mm./hr) have a small increase in protein-bound carbohydrates; this increase, evaluated from Fig. 2, would perhaps be connected with a gamma-globulin increase rather than with an alpha-globulin increase, an assumption that would fit with the low grade chronic type of the osteo-arthritis disease process.

Thus, no *qualitative* differences were found in the values for protein-bound carbohydrates in the various forms of collagen disease, although quantitatively the total amounts varied, probably to a large extent because of variations in disease activity. These problems will be discussed in a following paper (Böttiger and others, 1964).

To see whether differences could be found in single protein fractions, sera from RA and SLE patients were submitted to zone electrophoretic separations in polyvinyl chloride, and protein and

protein-bound carbohydrates were determined in the fractions obtained. In the seven sera analysed, all taken from patients with very active disease, the protein pattern in RA differed from that in SLE (Table III; Figs 3 and 4). The RA cases had increased α_1 and α_2 globulins as the dominant finding. The increase in alpha-globulins was also found in the SLE patients, but their chief characteristics were the high gamma globulin and low albumin levels. This difference between RA and SLE becomes especially evident if an α_2 /gamma-ratio is calculated, the value being much lower in SLE (0.33) than in RA (0.68). The same difference is also found in the series as a whole (cf. Table II) (SLE 0.34 and RA 0.50). Heiskell, Carpenter, Weiner, and Nakagawa (1961) reported similar findings.

No differences were found in the carbohydrate content of the different protein components. Table IV shows that the amount of bound carbohydrate is very constant and is the same in RA and SLE patients and in normal subjects. This is true not only for the sum of the carbohydrates as shown in the Table, but also for the single components (hexoses, hexosamines, and sialic acids). Südhof, Kruppa, and Thum (1959), using a cellulose acetate "paper" as supporting medium with a following PAS-staining, reported a lowered carbohydrate content in the gamma globulin fraction of patients with RA as compared with healthy individuals. This finding was not verified by our study. On the contrary, all experience in our laboratory speaks in favour of constant protein-carbohydrate ratios for all the major serum globulin fractions, although the absolute amount of the fractions may vary widely in various diseases.

There is nothing to support a theory—or a hope—that differentiated analyses of protein-bound carbohydrates, in whole serum or after zone electrophoresis, may be of value in the differential diagnosis between the collagen disease. This, of course, does not imply that "specific" protein or protein-carbohydrate complexes do not exist. It can only be stated that such components do not occur in amounts large enough to be detected by the chemical methods used.

Summary

Protein-bound carbohydrates (hexoses, hexosamines, and sialic acids) were analysed in whole serum from 187 patients and, after zone electrophoretic separation in seven patients, with rheumatic disorders. The results demonstrated a

considerable increase in the protein-bound carbohydrates in all the diseases tested. No specific changes in the occurrence of protein-bound carbohydrates were found, all electrophoretic separations showing a constant carbohydrate protein ratio.

Such analyses do not seem to be of value in the differential diagnosis between the rheumatic disorders.

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Les hydrates de carbone liés aux protéines dans les maladies rhumatismales

I. Résultats des analyses différentielles dans plusieurs désordres rhumatismaux

RÉSUMÉ

Les hydrates de carbone liés aux protéines (hexoses, hexosamines et acides sialiques) furent analysés dans le sérum complet de 187 sujets atteints de différentes maladies rhumatismales. Dans 7 cas d'arthrite rhumatismale sévère ou de lupus érythémateux disséminé on étudia la fraction protéique après l'avoir séparé par l'électrophorèse. Dans toutes les maladies étudiées on nota une augmentation considérable des hydrates de carbone liés aux protéines mais on n'y vit pas d'altérations spécifiques. Toutes les séparations électrophorétiques montrèrent l'existence d'un rapport constant entre les hydrates de carbone et les protéines.

De telles analyses ne semblent avoir aucune valeur dans le diagnostic différentiel entre les maladies rhumatismales.

Los carbohidratos ligados con proteínas en las enfermedades reumáticas

I. Resultados de analisis diferenciales en varios disturbios reumáticos

SUMARIO

Los carbohidratos ligados con proteínas (hexosas, hexosaminas y ácidos sialicos) fueron analizados en el suero completo de 187 sujetos con diferentes enferme-

dades reumáticas. En 7 casos de artritis reumatoide grave o de lupus eritematoso diseminado se estudió la fracción protéica después de separarla por electroforesis. En todas las enfermedades estudiadas se notó un aumento considerable de carbohidratos ligados con proteínas pero no se vieron alteraciones específicas de éstos. Todas las separaciones electroforéticas mostraron la existencia de una razón constante entre los carbohidratos y las proteínas.

Analisis de este tipo no parece tener valor alguna en el diagnóstico diferencial entre los disturbios reumáticos.

SERUM PROTEIN-BOUND CARBOHYDRATES IN RHEUMATIC DISEASE

II. EVALUATION OF ACTIVITY IN RHEUMATOID ARTHRITIS

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The evaluation of activity in rheumatoid arthritis has long been a problem for the clinician and no final solution has yet been reached. Many different reactions have been used, the most common being the erythrocyte sedimentation rate (ESR), tests for C-reactive protein (CRP), electrophoretic determination of α_2 -globulins, and chemical analysis of various serum protein-bound carbohydrates, especially non-glucosamine polysaccharide (Shetlar, Payne, Padron, Felton, and Ishmael, 1956), seromucoid (Winzler, 1955; Nettelblatt and Sundblad, 1962), and lately also haptoglobin (Nettelblatt and Sundblad, 1962; Müller, Kluthe, and Müller, 1963). The problems involved have been discussed *inter alia* by Shetlar and others (1956), Stidworthy, Payne, Shetlar, and Shetlar (1957), Nettelblatt and Sundblad (1962), Voit and Gamp (1962), and Müller and others (1963).

We have studied a group of 61 patients with rheumatoid arthritis to discover whether differentiated analyses of serum protein-bound carbohydrates might be of value in assessing the activity of the disease process, and whether such analyses would be better than the more simple tests used previously. Analyses have been made of protein-

bound carbohydrates (hexoses, hexosamines, and sialic acids) as well as of two carbohydrate-rich "proteins", seromucoid and haptoglobin.

Material

The series comprised 61 persons, mainly out-patients, from the Department of Rheumatology, Karolinska Sjukhuset. All fulfilled the diagnostic criteria of rheumatoid arthritis set up by Ropes, Bennett, Cobb, Jacox, and Jessar (1959). The rheumatoid factor test was positive in 53 of them (87 per cent.).

The activity of the disease was clinically evaluated by one of the authors (E.M.) while unaware of the results of the chemical analyses:

Group 1 (slight or none).—Six patients with a history of morning stiffness, swollen joints, or pain on movement without *present* signs of arthritis but with a positive rheumatoid factor test. A few patients with transient swelling of single joints were also included in this group.

Group 2 (mild).—26 patients with swelling of one or more joints, remaining from one examination to the other, or widespread old rheumatoid arthritis, possibly with marked deformities, but without actual swelling of the capsules or exudative joint changes.