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

BY

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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 alpha₂-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.

Group 3 (high).—27 patients with widespread joint disease, with swelling, exudates, and pain on movement, and often with slight elevation of body temperature.

Group 4 (severe).—Two patients as in Group 3, but with a definite increase of temperature in the diseased joints and of body temperature.

(These criteria generally coincide with those used by Shetlar and others, 1956.)

Methods

Protein-bound Carbohydrates were determined by the methods previously described (Böttiger and Carlson, 1960).

ESR—Westergren.

Sheep Cell Agglutination Test (S.C.A.T.).—Svartz and Schlossmann (1952).

Paper Electrophoresis.—In barbital buffer pH 8.6, ionic strength 0.1. The strips were stained with bromophenol blue and evaluated by elution of the dye.

Total Protein.—A biuret method.

Seromucoid.—The method of Winzler (1955), with the modifications described by Böttiger and Carlson (1960).

Haptoglobin.—Jayle's peroxidase method as described by Nyman (1959).

Results

The results are given in Table I. The percentage increase from normal levels with increasing activity was calculated (Figure, opposite); the variables increase with increasing activity of the disease. To test their relative value in distinguishing between normal subjects and rheumatoid arthritics with low

disease activity and between arthritis with low and high disease activity, the differences have been calculated between the mean values and the significance of the differences tested (Table II).

TABLE II

Analysis		Disease Activity	
		Low compared with Normal Subjects	High compared with Low Degree of Rheumatoid Arthritis
Protein-bound Carbohydrates	Hexoses ..	xxx	x
	Hexosamines ..	xxx	xx
	Sialic acids ..	xxx	xx
Protein Fractions	Albumin ..	xx	—
	alpha ₂ globulin ..	xxx	—
	gamma globulins ..	xxx	—
Seromucoid ..		—	xx
Haptoglobin ..		xxx	—
Haemoglobin ..		xxx	—
Erythrocyte Sedimentation Rate (Westergren) ..		xxx	xxx

The significance of the difference was tested by the "t-test".

P values indicated in Table as follows:

P > 0.5 = —
 0.01 < P < 0.05 = x
 0.001 < P < 0.01 = xx
 P < 0.001 = xxx

Discussion

The first problem that arises in studies of this type is: How to do a clinical grading of activity that can serve as a baseline for an assessment of the value of chemical tests for evaluating the same factor, i.e. activity. What clinical criteria are the best and most reliable ones? Many attempts have been

PROTEIN-BOUND CARBOHYDRATES, PAPER ELECTROPHORESIS, SHEEP CELL AGGLUTINATION TEST, ARTHRITIS OF VARYING ACTIVITY

Disease Activity Group	No. of Cases	Protein-bound Carbohydrates (mg./100 ml.)				Seromucoid* (mg./100 ml.)	Haptoglobin (mg./100 ml.)
		Hexoses	Hexosamines	Sialic Acids	Total		
1	6	126 ± 7 16	106 ± 7 16	67 ± 5 13	301 ± 18 45	—	—
2	26	140 ± 5 25	113 ± 4 19	77 ± 3 14	334 ± 11 54	97 ± 8.6 33 (n = 15)	201 ± 18 94
3	27	152 ± 3 17	125 ± 2 12	85 ± 2 11	362 ± 7 37	135 ± 8.5 33 (n = 15)	241 ± 14 71
4	2	166	165	126	456	—	—
Normal Subjects ..	10	112 ± 0.8	93 ± 0.6	67 ± 0.4	270 ± 1.6	86 ± 2.0	110 ± 2.0

Mean values ± standard error of the mean and (below) standard deviations are given.

* Seromucoid analyses were not performed on all sera; actual number of tests in brackets.

Figure.—Percentage increase in the results of various tests in patients with low and high activity rheumatoid arthritis, compared with normal values

made to answer this question, most of them based upon or similar to the grouping used by Shetlar and others (1956). As pointed out by Kellgren (1960), Lansbury (1958), and most recently by Voit and Gamp (1962), such factors as the number and size of the joints involved may also be of importance, especially in studies of chemical variables produced by inflammation. The duration of disease is also important in considering gamma globulin levels, which may also be influenced by previous or recent therapy. Most of the classifications used depend on four activity groups, some have five or six, but

it is always difficult to get reliable numerical estimations of activity which are suitable for use in regression analysis. We have chosen to exclude the two extreme groups (Numbers 1 and 4 in the Table) which contain only a few patients, and most of the following comparisons will be made between normal subjects and the arthritics in the “low” and “high” activity groups (Groups 2 and 3, Table I).

Table I and the Figure show that all the variables studied increase with increasing activity of the rheumatic process. The only striking exception is the sheep cell agglutination test, which shows no

ERYTHROCYTE SEDIMENTATION RATE, AND HAEMOGLOBIN IN 61 PATIENTS WITH RHEUMATOID AND TEN NORMAL SUBJECTS

Protein—Paper Electrophoretic Fractions† (g./100 ml.)			ESR (Westergren) (mm./hr)	Haemoglobin (g./100 ml.)	Sheep Cell Agglutination Test
Albumin	alpha ₂ -globulin	gamma-globulin			
4.43 ± 0.16 0.39	0.66 ± 0.06 0.13	1.50 ± 0.10 0.22	17 ± 5 12	11.8 ± 0.35 0.85	1/128 —
4.41 ± 0.12 0.55	0.78 ± 0.03 0.13	1.60 ± 0.10 0.41	27 ± 5 20	11.5 ± 0.49 1.20	1/512 —
(n = 17)					
4.25 ± 0.08 0.40	0.83 ± 0.03 0.12	1.66 ± 0.06 0.28	39 ± 3 17	11.1 ± 0.20 1.03	1/256
(n = 22)					
3.74	1.45	1.96	103	10.2	1/1,024
4.82 ± 0.09	0.50 ± 0.02	1.08 ± 0.04	15 ± 1.5	15.0 ± 0.10	—

† Paper electrophoresis was not performed on all sera; actual number of analyses given in brackets.

differences between the two groups under discussion. It was pointed out by Svartz and Schlossmann (1952) that the amount of rheumatoid factor present showed only a weak correlation with clinical activity, a fact later emphasized by Shetlar and others (1956).

The Figure attempts to show which test should be given preference as a clinical tool in evaluating disease activity. The mean percentage increase of the variables from normal levels (listed at the bottom of Table I) have been calculated. More or less "straight" plots are found for the erythrocyte sedimentation rate and protein-bound carbohydrates, indicating that these variables would distinguish between normal subjects and patients with low activity as well as between patients with low and with high activity. Statistical analysis of the differences between the means for the absolute values supports this finding. Table II shows that the best differentiation in both steps is achieved by the ESR, the protein-bound carbohydrates giving a slightly lower degree of separation between patients with low and high activity of disease.

The angled plots in the Figure indicate that certain procedures do not distinguish between degrees of disease activity. For example, seromucoid does not separate low activity patients from normal subjects, and the α_2 -globulins and haptoglobin do not separate low activity from high activity patients.

The variables analysed are closely interrelated. ESR is well correlated with the total amount of protein-bound carbohydrates (compare Böttiger, Malmquist, and Olhagen, 1964). The carbohydrates themselves (hexoses, hexosamines, and sialic acids) occur in constant molecular ratios (Böttiger, Estborn, and Möllerberg, 1960). The protein fractions richest in carbohydrates are the α_2 -globulins, of which haptoglobin constitutes the largest part. The calculation of correlation coefficients fully confirms this intimate relationship.

From a theoretical point of view, therefore, the method chosen is immaterial, and the choice depends upon the accuracy of the method, its simplicity, and the normal variations in the results. Seromucoid and haptoglobin determinations are rather complicated, their normal variations are large (the ± 2 S.D. values being 44-128 and 28-192 mg./100 ml., respectively), and their differentiating capacity is limited. The values for protein-bound carbohydrates have a much smaller range of normal variation and the experimental errors are extremely small, especially for sialic acid and hexosamine. The methods are not easy, however, especially when compared with the ESR, which combines extreme

simplicity with the most precise differentiating capacity. We therefore recommend the ESR for general use and the determination of protein-bound sialic acids or hexosamines, if an additional criterion is needed.

How does this agree with previous experience? Shetlar and others (1956) advocated the determination of protein-bound hexoses, hexosamines were found valuable by Gamp (1962), and sialic acid determinations by various methods were found useful by some authors (Gamp, 1962; Adam, Malecek, and Kútová, 1957; Müller and others, 1963) and less so by others (Nettelblatt and Sundblad, 1962). Seromucoid was found to be best by Nettelblatt and useful by Gamp, and haptoglobin was found useful by Nettelblatt, by Müller, and by Gamp.

Müller and others (1963) also tried ceruloplasmin as one of the α_2 components, but found it had no advantages over other methods.

Finally, Shetlar and others (1956) found only a borderline correlation between ESR and clinical activity, whereas Gamp (1962) and Müller and others (1963) consider the ESR to be the method of choice. This discrepancy may be due to the ESR's having been estimated by different techniques. Shetlar and others (1956) used the Wintrobe method, whereas those who found it more sensitive used the Westergren method.

Our patients were mainly early and mild cases, but Strandberg (1964) analysed another and larger group of hospitalized patients, with more severe arthritis. He found a slightly higher ESR and lower albumin values, but nothing to support the idea of any essential difference between the two groups. Analyses of haemoglobin and albumin are included in our Tables; they both decrease with increasing activity of the disease. Their relative value as criteria is discussed by Strandberg (1964).

CRP determinations have been widely used as a measure of activity, and there is general agreement upon the fact that severe inflammatory reactions are followed by an elevated CRP-level. The test has some disadvantages, and is said not to correlate well with low activity (Shetlar and others, 1956; Nettelblatt and Sundblad, 1962). On the other hand, it has also been found to be too sensitive, as it may become positive after a diagnostic aortography (Ljunggren). Furthermore, it is only a semi-quantitative method and, although it is technically easy to perform, methodological errors must also be taken into consideration (Shetlar and others, 1956). These considerations made us decide not to include the CRP-test in the present study.

Summary

Protein-bound carbohydrates (hexoses, hexosamines, and sialic acids), seromucoid, haptoglobin, paper electrophoresis of globulin fractions, the sheep cell agglutination test, and the erythrocyte sedimentation rate have been studied in a series of 61 patients with rheumatoid arthritis, in an attempt to correlate the degree of clinical activity with the results of the various tests. The study indicates that the ESR is still the method of choice.

REFERENCES

- Adam, M., Malecek, J., and Kútová, M. (1957). *Ann. rheum. Dis.*, **16**, 69.
- Böttiger, L. E., and Carlson, L. A. (1960). *Clin. chim. Acta*, **5**, 664.
- , Estborn, B., and Möllerberg, H. (1960). *Acta Soc. Med. upsalien.*, **65**, 72.
- , Malmquist, E., and Olhagen, B. (1964). *Ann. rheum. Dis.*, **23**, 489.
- Gamp, A. (1962). *Z. Rheumaforsch.*, **21**, 128.
- Kellgren, J. H. (1960). In "Controlled Clinical Trials, Conference convened by the Council for International Organizations of Medical Sciences, Vienna, 1959", p. 69. Blackwell, Oxford.
- Lansbury, J. (1958). *Arthr. and Rheum.*, **1**, 505.
- Müller, W., Kluthe, R., and Müller, H. (1963). *Z. Rheumaforsch.*, **22**, 1.
- Nettelblatt, E., and Sundblad, L. (1962). *Acta rheum. scand.*, **8**, 57.
- Nyman, M. (1959). *Scand. J. clin. Lab. Invest.*, Suppl. 39.
- Ropes, M. W., Bennett, G. A., Cobb, S., Jacox, R., and Jessar, R. A. (1959). *Ann. rheum. Dis.*, **18**, 49.
- Shetlar, M. R., Payne, R. W., Padron, J., Felton, F., and Ishmael, W. K. (1956). *J. Lab. clin. Med.*, **48**, 194.

- Stidworthy, G., Payne, R. W., Shetlar, C. L., and Shetlar, M. R. (1957). *J. clin. Invest.*, **36**, 309.
- Strandberg, O. (1964). To be published.
- Svartz, N., and Schlossmann, K. (1952). *Acta med. scand.*, **142**, 420.
- Voit, K., and Gamp, A. (1962). *Z. Rheumaforsch.*, **21**, 3.
- Winzler, R. J. (1955). *Meth. biochem. Anal.*, p. 279.

Les hydrates de carbone liés aux protéines dans les maladies rhumatismales

II. Evaluation de l'activité morbide de l'arthrite rhumatismale

RÉSUMÉ

Les hydrates de carbone liés aux protéines (hexoses, hexosamines et acides sialiques), les séromucoïdes, l'haptoglobuline, l'électrophorèse sur papier des fractions globuliniques, la réaction de Waaler-Rose et la vitesse de sédimentation globulaire furent étudiés dans une série de 61 sujets atteints d'arthrite rhumatismale pour mettre en corrélation l'intensité de l'activité morbide avec les résultats de différents tests. On en conclut que la vitesse de sédimentation globulaire est toujours le procédé de choix.

Los carbohidratos ligados con proteínas en las enfermedades reumáticas

II. Valoración de la actividad morbosa en la artritis reumatoide

SUMARIO

Carbohidratos ligados con proteínas (hexosas, hexosaminas y ácidos sialicos), seromucoides, heptaglobulina, electroforesis sobre papel de fracciones globulinicas, reacción de Waaler-Rose y velocidad de sedimentación eritrocitaria fueron estudiados en 61 enfermos con artritis reumatoide para correlacionar el grado de actividad morbosa con los resultados de varios tests. Este estudio indica que la velocidad de sedimentación eritrocitaria aún queda el método de elección.