ON THE NATURE OF THE RHEUMATOID FACTOR*

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The researches initiated by Cecil, Nichols, and Stainsby (1931), Waaler (1940), and Rose, Ragan, Pearce, and Lipman (1949) on the agglutinating activity of serum from patients with rheumatoid arthritis led to the discovery of a serum factor, now regarded as characteristic of rheumatoid arthritis, and therefore designated "rheumatoid factor" (Pike, Sulkin, and Coggeshall, 1949; Svartz, 1956, 1959). This factor is important not only in differential diagnosis but also because it may take part in the pathogenesis of rheumatoid arthritis.

The rheumatoid factor has been located in the globulin fraction (Waaler, 1940), in Cohn's "Fraction III" (Heller, Kolodny, Lepow, Jacobson, Rivera, and Marks, 1955) and in the euglobulin fraction (Lamont-Havers, 1955; Svartz, Carlson, Schlossmann, and Ehrenberg, 1958; Svartz and Schlossmann, 1955); electrophoretically, it migrates with the gamma-globulin.

With the ultracentrifuge, the gamma-globulin fractions of normal serum can be shown to consist of two components, one having a sedimentation constant of 19 Svedberg units (19 S gamma-globulin) and a molecular weight of about 900,000, the other having a sedimentation constant of 7 Svedberg units (7 S gamma-globulin) and a molecular weight of about 156,000 (Cohn, 1947; Müller-Eberhard, Kunkel, and Franklin, 1956; Müller-Eberhard, Franklin, and Kunkel, 1957; Pedersen, 1945; Wallenius, Trautman, Kunkel, and Franklin, 1957). The 19 S fraction amounts to only 5 per cent. of the total gamma-globulin. Müller-Eberhard (1959) and Schultze (1957) have demonstrated that the carbohydrate content of the 19 S component is four times higher than that of the 7 S component.

In the sera from rheumatoid patients, a com-

ponent having a sedimentation constant of 22 Svedberg units has been detected (Fig. 1) (Franklin, Holman, Müller-Eberhard, and Kunkel, 1957; Franklin, Kunkel, Müller-Eberhard, and Holman, 1957; Franklin and Kunkel, 1957; Müller-Eberhard and others, 1956). Franklin and Müller-Eberhard and their co-workers have shown that this component is responsible for the positive agglutination reaction. They also demonstrated that the 22 S component represents a complex of 19 S and 7 S gamma-globulin in the ratio of 43 : 57 per cent.; and they conclude that there is one heavy for every six light molecules of gamma-globulin. Serological activity thus appears to be characteristic of the 19 S fraction.

Fig. 1.—Comparison of ultracentrifugal patterns in normal and rheumatoid serum:

- (a) Serum from a patient with rheumatoid arthritis, showing two components which sediment quickly, the physiological 19-S fraction and the pathological 22-S;
- (b) Normal serum under same conditions (according to Müller-Eberhard, Kunkel, and Franklin, 1957).

Studies utilizing zone electrophoresis, chromatography in cellulose columns and ultracentrifugation confirm that the rheumatoid factor is a macromolecular gamma-globulin of the 19 S type with

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a molecular weight of 900,000 (Franklin and others, 1957; Müller-Eberhard and others, 1956, 1957; Svartz, 1956, 1959; Svartz and others, 1955, 1958). According to Svartz (1959), it contains approximately 9 per cent. carbohydrate.

Apart from its serological reactivity the rheumatoid 19 S gamma-globulin reacts physically and chemically in the same way as normal 19 S gamma-globulin (Müller-Eberhard, 1959; Müller-Eberhard and others, 1957). Müller-Eberhard (1959) believes however, that a difference in molecular structure may account for the serologic reactivity.

It was therefore considered worth while to carry out a detailed chemical analysis of the rheumatoid factor.

Method

Pure rheumatoid factor was obtained by precipitation with gamma-globulin in the following way:

Serum from rheumatoid patients having high titres in erythrocyte and latex-fixation tests was mixed in 50 ml. aliquots with the same volume of a solution of gamma-globulin* and the mixture was added to borate buffer (pH 8.0) to give a concentration of 0.5 per cent. After 2 hours at room temperature and a further 46 hours at 4° C., the resultant precipitate was separated by centrifugation at 17,000 r.p.m. for 20 min. The precipitate was washed three times with distilled water and dried to a constant weight over concentrated sulphuric acid. To obtain a sufficient amount of precipitate, serum samples from five different patients were pooled.

The experiments of Müller-Eberhard have shown that the 7 S and 19 S fractions (Fig. 2) are in a ratio of 1 : 1 in a solution of the precipitate in the ultracentrifuge; aggregates with 35 S and 150 S were also observed

Fig. 2.—Analytical ultracentrifugal diagram of a precipitate solved in 5-M urea, which was gained by charging the serum of a rheumatoid patient with gamma globulin heated for a short period. The sedimentation coefficient of the quick component amounts to 19 S and that of the slow one to 7 S.

* Behringwerke, Marburg.

which derived from the 7 S gamma-globulin added for the precipitation. According to a personal communication from Müller-Eberhard, the gamma-globulin aggregates were found to amount to 50-70 per cent. of the total precipitate if the gamma-globulin was first heated. In the present experiments the gamma-globulin has not been heated. A solution of the precipitate in 5-M urea was repeatedly centrifuged at 20,000 r.p.m. for periods of up to 3 hours and the above mentioned aggregates were found to comprise 40 per cent. of the total precipitate. In repeated tests this component showed no serological activity. It was concluded that 70 per cent. of the precipitate consists of 7 S gamma-globulin, the remainder being 19 S gamma-globulin and corresponding to the rheumatoid factor. This ratio permits the two components to be estimated separately.

Colorimetric analyses of the carbohydrate were carried out. Hexoses were determined by the methods of Holzman, MacAllister, and Niemann (1947) and Winzler (1955); fucose by the method of Jacubeit, Brünger, and Knedel (1959); hexosamine by that of Dische and Borenfreund (1950); neuramine (sialic acid)† by that of Schultze, Schmidtberger, and Haupt (1958); the polysaccharide was estimated by the tryptophane reaction (Shetlar, Foster, and Everett, 1948) and the total nitrogen by the method of Kjeldahl (Hallmann, 1950). The amino acids were determined by the method of Moore and Stein (1948) after protein hydrolysis (Zahn, 1954), and elution from a two-way paper chromatogram (Levy and Chung, 1953).

Results

Table I (opposite) shows the carbohydrate content of the rheumatoid factor, and normal 7 S and 19 S gamma-globulin.

The polysaccharide content of 7 S found by Müller-Eberhard and his co-workers (1956, 1957, 1959) is relatively low; the hexoses seem to be highest followed by hexosamine, fucose, and neuramine in that order. By comparison with 7 S, the normal 19 S fraction presents a threefold increase in the polysaccharide content, a fourfold increase in hexoses, a twofold increase in fucose, and a sevenfold increase in neuramine.

The total carbohydrate content of the rheumatoid factor (10.78 per cent.) found by us is higher than the 9 per cent. reported by Svartz (1959). Our results for the carbohydrate components of the rheumatoid factor seem to correspond closely with those obtained by Müller-Eberhard for normal 19 S gamma-globulin.

Corresponding with the increased carbohydrate content, the total nitrogen content of the rheumatoid

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TABLE I

CARBOHYDRATE CONTENT OF RHEUMATOID FACTOR (FRACTION $S_{20} = 19$ S) COMPARED WITH
NORMAL GAMMA GLOBULIN (FRACTIONS $S_{20} = 7$ S AND $S_{20} = 19$ S)

Fraction		Normal Gamma Globulin				Rheumatoid Factor
		$S_{20} = 7$ S		$S_{20} = 19$ S		$S_{20} = 19$ S
Authors and Dates		(Müller-Eberhard, 1959)	(Wöhler and others, 1960)	(Müller-Eberhard, 1959)	(Schultze)	(Wöhler and others, 1960)
Carbohydrate Content (per cent.)	Hexose	1.22	1.23	5.2	4.16	5.13
	Fucose	0.29	0.3	0.62	0.46	0.85
	Hexosamine ..	1.14	1.2	2.9	2.4	2.9
	Neuramine Acid	0.22	0.25	1.7	1.81	1.84
	Polysaccharide	2.58	2.7	9.8	8.83	10.78

factor is smaller than the 7 S gamma-globulin, 14.7 g. per cent. as against 16.03 g. per cent. (Table II, overleaf).

When the two-way paper chromatography was applied after hydrolysis of protein, a large series of amino acids could be proved (Fig. 3).

The quantitative values of the amino acids in the rheumatoid factor and in 7 S gamma-globulin are shown in Table II. We used the amino acid composition of 7 S gamma-globulin (Brand, 1946; Brand, Kassell, and Saidel, 1944) for comparison because, according to Franklin and others (1957), Heimer (1959), and Heimer and Federico (1958), decomposition of the intermolecular S-S

links of the rheumatoid factor is accompanied by a depolymerization into units of 7 S gamma-globulin; repolymerization may be prevented by adding moniodine acetate (Islaker, 1958; Heimer, 1959; Heimer and Federico, 1958).

Glutamic acid comes first with about 13 per cent., followed by aspartic acid, valine, threonine, lysine, proline, leucine, serine, tyrosine, and so on. While in general the values seem to be somewhat lower in the rheumatoid factor, the values in the rheumatoid factor for cysteine and cystine are higher than in the 7 S gamma-globulin. The higher cystine value may be an expression of the increased S-S links which might be expected from the findings of Franklin and

Fig. 3.—Two-way chromatogram of the rheumatoid factor.

Presentation of the amino acids with ninhydrine:

Solvent I: Isopropanolformic acid-glacial acetic acid-water,
500 : 105 : 105 : 105;

Solvent II: Phenol-water mixture, 5 : 1.

TABLE II

COMPOSITION OF AMINO ACIDS, AMOUNT OF NITROGEN, AND MOLECULAR WEIGHT OF RHEUMATOID FACTOR (S 19 FRACTION) COMPARED WITH NORMAL GAMMA GLOBULIN (S-7 FRACTION)

Globulin Fraction	Normal Gamma Globulin (S ₂₀ = 7 S)	Rheumatoid Factor (S ₂₀ = 19 S)
Molecular Weight	(Cohn, 1957) 156,000	(Müller-Eberhard, 1959) 900,000
Total Nitrogen (per cent.)	(Brand, 1946) 16.03	(Wöhler and others, 1960) 14.7
Amino Acids (per cent.)	(Brand, 1946)	(Wöhler and others, 1960)
Glycine	4.2	3.6
Alanine	2.2	2.0
Valine	9.7	8.2
Leucine	9.3	7.3
Isoleucine	2.7	2.3
Proline	8.1	7.6
Phenylalanine	4.6	4.5
Cysteine	0.7	0.99
Cystine	2.37	3.4
Methionine	1.06	1.1
Tryptophan	2.86	2.7
Arginine	4.8	4.2
Histidine	2.5	3.0
Lysine	8.1	7.7
Aspartic acid	8.8	9.6
Glutamic acid	11.8	13.1
Serine	11.4	7.3
Threonine	8.4	8.2
Tyrosine	6.75	7.0

others (1957) regarding depolymerization of the rheumatoid factor. Although the amino acid percentage composition of the rheumatoid factor and normal 7 S gamma-globulin appear to be similar, one cannot be certain that the quantitative values are identical.

The present results have enabled the carbohydrate content and the amino acid composition of the rheumatoid factor to be established. Since its carbohydrate content resembles that of the normal 19 S gamma-globulin fraction and its amino acids correspond with 7 S gamma-globulin, this protein and 19 S may be closely related. Which physico-chemical correlate the serological activity is based on cannot be decided. Examination of the active end-groups might help to elucidate this problem.

Summary

Chemical analyses have been made on the rheumatoid factor isolated by precipitation with gamma-globulin. The carbohydrate content of the rheumatoid factor is similar to that of normal 19 S gamma-globulin: hexose 5.13 per cent., fucose 0.85 per cent., hexosamine 2.9 per cent., and neuramine 1.84 per cent. The percentage amino acid composition of the rheumatoid factor was similar to that of normal 7 S gamma globulin.

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Sur la nature du facteur rhumatismal

RÉSUMÉ

Le facteur rhumatismal, isolé par précipitation avec la globuline gamma, fut soumis à l'analyse chimique. Le contenu en hydrates de carbone du facteur rhumatismal est similaire à celui de la globuline gamma 19 S normale: hexose 5,13%, fucose 0,85%, hexosamine 2,9% et neuramine 1,84%. Le pourcentage des amino-acides dans le facteur rhumatismal fut similaire à celui de la globuline gamma 7 S normale.

Sobre la naturaleza del factor reumatoide

SUMARIO

El factor reumatoide, aislado por precipitación con la globulina gama, fue sometido al análisis químico. El contenido en hidratos de carbono del factor reumatoide es similar al de la globulina gama 19 S normal: hexosa 5,13%, fucosa 0,85%, hexosamina 2,9% y neuramina 1,84%. La proporción de los amino-ácidos en el factor reumatoide fue similar a la en la globulina gama 7 S normal.