ON THE NATURE OF THE RHEUMATOID FACTOR

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It is known that in association with certain chronic illnesses (infectious nonspecific polyarthritis, disseminated lupus erythematosis, scleroderma, nodular polyarthritis, etc.) there arise clearly manifested changes in the composition of the serum proteins, which are characterized by an increase in the γ-globulins and the appearance of the so-called macroglobulins in the blood [3, 7, 13, et al.]. It has been established that the serum of these patients in many cases acquires the property of agglutinizing sheep erythrocutes sensitized with a non-agglutinizing dose of the corresponding immune serum. The agglutinizing factor is most frequently observed in the serum of patients with infectious nonspecific polyarthritis or rheumatoid arthritis, using the nomenclature of the English and American authors [1-5, 12, 14, 16, 17, 18, et al.].

The agglutinizing or rheumatoid factor in the serum of patients with infectious nonspecific polyarthritis was discovered by Waaler [16], following which Rose and coworkers established the possibility of using this test diagnostically [12]. At the present time the so-called Waaler-Rose reaction has obtained wide acceptance as a method for the laboratory diagnosis of infectious nonspecific polyarthritis. In addition, this technique has been modified, and other methods have been advanced for demonstrating the rheumatoid factor [6, 8, 14, 18].

Studying the properties of the rheumatoid factor showed that it is resistant to heating at 56° C for 30 minutes [16], to the action of alcohol and ether [11], papain and trypsin; it is inactivated at 80° C and pH 2 [18]. Through an investigation involving prolonged rapid centrifugation it was established that the rheumatoid factor is contained in the fractions with a sedimentation coefficient of 19-22 S, wherein the fraction with a coefficient of 22 S appears to be a complex of components of both 19 and 7 S [13]. The agglutinizing activity appears to be in the protein fractions characterized as γ-globulins [10, 15, 17, 18], which is supported by electrophoretic and chromatographic investigations [13, 18]. However, there are data indicating that the rheumatoid factor is also contained in the β-globulin fraction [12, 18]. Thus the question of the nature of the rheumatoid factor remains unresolved, and further study in this area is necessary.

In this report we present the results of a comparative study on the content of rheumatoid factor in the γ-globulin fractions obtained by various methods.

EXPERIMENTAL METHOD

Sera from patients with infectious nonspecific polyarthritis were tested for the presence of rheumatoid factor by the agglutination reaction with sensitized sheep erythrocytes [8, 14]. The serum was first inactivated by heating at 56°C for 30 minutes and absorbed on sedimented sheep red blood cells in order to remove the normally present heterophilic antibodies. The agglutinizing activity of the rheumatoid factor was determined in test tubes, 0.5 cm in diameter and 1.2 cm in height. To two drops of serum, in various dilutions, we added one drop of sheep red blood cells, sensitized by the usual hemolytic serum used for the reaction of complement fixation. The serum was first tested for its content of agglutinizing antibodies against sheep erythrocytes, and used in a dilution exceeding its agglutinizing titer by four times. For the sensitization, the sheep erythrocytes, in the form of a 3% suspension, were mixed with an equal volume of hemolytic serum in the necessary dilution, and incubated for 30 minutes at 37° C. A mixture of the test serum and the sensitized erythrocytes was placed in an incubator at 37° for 1 hour, and then in a refrigerator until morning of the following day. The reaction was appraised according to the configuration of the sediment, using a hand lens.

For control purposes the serum was tested with a 1.5% suspension of nonsensitized erythrocytes. The result was considered positive if, in the absence of a reaction in the control, the test serum agglutinized the sensitized erythrocytes in a dilution of 1.16 and higher. Sera with an agglutinizing titer of 1:32 and higher underwent further treatment, allowing us to obtain the protein fractions with the high γ-globulin content (they will be subsequently designated as the A, B, and C fractions).

In obtaining the A fraction we used the method of Wolfson and coworkers, adapted by Whillans and Fischman [17] for extraction of euglobulin containing the rheumatoid factor. In this method, 193 grams of ammonium sulfate are dissolved in 1 liter of a 4% solution of sodium chloride. One volume of the serum is added to 24 volumes of the solution obtained, the mixture is stirred for 2 minutes and centrifuged for 30 minutes at 2500 - 3000 rpm. The supernatant fluid is poured off, the walls of the test tube are dried with a gauze tampon, and the sediment is dissolved in physiological saline, using an amount corresponding to the volume of the serum sample. We obtained the B fraction by treating fraction A with a 0.4% solution of rivanol, following the method of Horejsi and Smetana [9]. To one volume we added $3\frac{1}{2}$ volumes of the rivanol solution. After 20 minutes we centrifuged the mixture for 10 minutes at 3000 rpm. The supernatant fluid was treated with activated carbon dust to remove the rivanol, and again centrifuged, this time at 2000 rpm for 10 minutes. The supernatant fluid was designated as fraction B.

To obtain fraction C we dissolved the precipitate that came down when we treated the A fraction with rivanol. The precipitate was mixed with activated carbon dust and physiological saline, the latter in an amount corresponding to the volume of the original solution of fraction A. The mixture was carefully stirred with a glass rod, and left in the refrigerator at 4% for 45-60 minutes; during this time the mixing was repeated 3-4 times. Then it was centrifuged for 10 minutes at 3000 rpm. The resultant opalescent fluid was designated as the C fraction.

TABLE Results Obtained in a Comparative Investigation of the Agglutinizing Activity of γ-Globulin Fractions

Serum sample No.	Agglutinizing titer			
	original serum	fraction A	fraction B	fraction C
1	1:32	1:32	$<1:4\frac{1}{2}$	1:32
2	1:512	1:512	$<1:4\frac{1}{2}$	1:512
3	1:32	1:32	$<1:4\frac{1}{2}$	1:32
4	1:256	—	$<1:4\frac{1}{2}$	—
5	1:512	1:512	—	1:512
6	1:512	1:512	—	1:512
7	1:512	—	—	1:1 024
8	1:128	—	—	1:64
9	1:32	—	—	1:32
10	1:512	—	—	1:256
11	1:512	—	—	1:512
12	1:128	—	—	1:128
13	1:64	—	—	1:32

Note. Dash mark means the test was not performed.

Fractions A, B, and C, as well as the original serum, were tested for their mobility in an electrical field, using an apparatus for paper electrophoresis. The fractionation was carried out in a veronal buffer (pH 8.6, ionization constant of 0.125) on a strip of paper 4 cm in width, using an emf of 120 v, for 18 hours.

EXPERIMENTAL RESULTS

The figure shows photographs and densimetric curves of the results obtained in electrophoresis of the original serum and the A, B, and C fractions (patient B-n, infectious nonspecific polyarthritis). The original serum and fractions A and C were applied to the paper in equal volumes, while $2\frac{1}{4}$ times more fraction B was used. In this case we calculated that the latter was a γ-globulin, diluted in the rivanol solution in a proportion of $1:4\frac{1}{2}$ as compared to the original serum. The mobility of the A, B, and C fractions corresponded to the mobility of γ-globulins (see figure).

The original serum and each of the fractions were investigated for the presence of the rheumatoid factor, following the method described above.

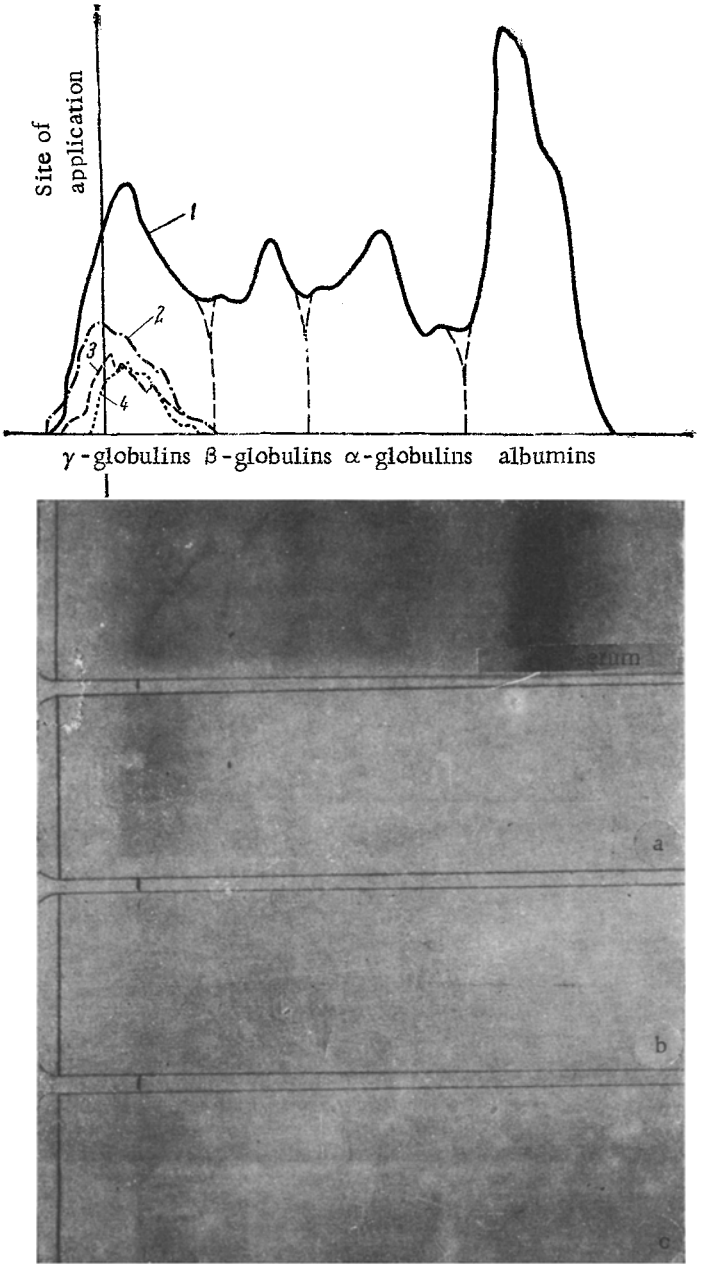
The table shows the results of examining 13 samples of serum from patients with infectious nonspecific polyarthritis, and the γ-globulin fractions from these sera.

As can be seen from the table, the agglutinizing activity of the tested serum samples ranged within dilutions of 1:32-1:512. A comparison of the results of determining the agglutinizing properties of the A, B, and C fractions shows that the capacity to agglutinize sensitized sheep erythrocytes is present in fractions A and C. The agglutinizing titer of these two fractions usually corresponded to the titer of the original serum. The exceptions consisted of samples No. 8, 10, 13, where we noted a reduction in the titer to the same degree, and sample No. 7, which showed a very small elevation in the agglutinizing titer. Fraction B (see table, samples No. 1, 2, 3, and 4),

representing a γ-globulin which does not precipitate in rivanol solution, was found to be inactive as far as its agglutinizing properties are concerned; this testifies to the absence of rheumatoid factor, or else to its low concentration.

Thus, the results of the investigations showed that the γ-globulin fractions of sera containing the rheumatoid factor differ in their agglutinizing activity. This fact should be taken into consideration in subsequent studies of the properties and biological nature of the rheumatoid factor.

It is interesting that the fraction which is not precipitated by rivanol, and is characterized by a high γ-globulin content, does not contain the agglutinizing factor. The latter is found in the fraction precipitated by rivanol, which indicates its link with other globulins.



Electrophoresis of the original serum and the A, B, and C fractions. Densimetric curves: 1) original serum; 2) fraction A; 3) fraction B; 4) fraction C.

SUMMARY

In the γ-globulin fraction obtained from the sera of patients with infectious nonspecific polyarthritis (rheumatoid arthritis) the rheumatoid factor level in the titer was 1.32-1:512, γ-globulin fractions A, B, and C confirmed by the results of electrophoresis on paper. The agglutinating factor in the titer corresponding to that of the initial serum was detected in the A fraction (obtained by the method of salting out with 19.3% ammonium sulphate in a 4% sodium chloride solution) and in the C fraction (fraction A component, precipitated as the results of the treatment of the former with a 0.4% rivanol solution). Fraction B, a component not precipitated from the serum with a 0.4% rivanol solution, produced no agglutinating activity. The described method of the C fraction isolation may be recommended for obtaining purified rheumatoid factor.

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All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.
