# SPECIFICITY OF ANTIGENIC COMPONENTS OF γ-GLOBULIN FORMED AFTER SPONTANEOUS BREAKDOWN OF THE MOLECULE

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Attention was first drawn by Grabard to the fact that the serum γ-globulins lose their homogeneity when stored in solution and form up to 3 precipitation lines during immunoelectrophoresis [1]. It was subsequently shown that after spontaneous breakdown of the molecule of Y-globulin at least 3 antigenic determinants may be found [3, 5, 14], and after enzymic hydrolysis from 3 to 5 different peptides may be formed, possessing different antigenic groups [4, 6, 13]. The idea thus took hold that the protein molecule may possess surface and internal, or latent, antigenic determinants. Attempts have now been made to identify the structure of the latent determinants of the protein molecule – albumins and γ-globulins [8, 9, 12]. The information which, in our opinion, deserves the most serious attention is the fact that after enzymic hydrolysis of rabbit γ-globulin, among the antigenic determinants characteristic of the untreated γ-globulin molecule other antigenic groups may be found, similar to α- and β-globulins [9].

We were interested to investigate the antigenic specificity of the fragments of human serum γ-globulin formed after spontaneous breakdown of the molecule during the process of storage of the protein in solution.

Comparison of the Precipitation Reaction of Different Antigammaglobulin Sera with γ-Globulins after Spontaneous Breakdown in the Process of Storage (Experiment on September 7-8, 1962)

| Human serum γ-globulins | | | Quantitative precipitation reaction | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Date of obtaining (1961) | Origin of blood serum | Period of storage (months) | No. of sera against γ-globulins | Vol. of antigen-antibody (in ml) | added γ-globulin (in mg/ml) | | | | | Number of components detected during immunoelectrophoresis |
| | | | | | 0.2 | 0.5 | 1 | 1.5 | 3 | |
| | | | | | amount of precipitate (in mg/ml) | | | | | |
| 3/5 | Patients C . . . . . | 18 | 75-C | 0.1 | 4.7 | 6.1 | 6.6 | 5.9 | 3.3 | 3 |
| 1/21 | Donors . . . . . | 19.5 | 75-C | 0.1 | 0 | 0.1 | 0.9 | 0.6 | 0.2 | 3 |
| 2/20 | Donors . . . . . | 18.5 | 75-C | 0.1 | 0.2 | 1.3 | 1.5 | 1.2 | 0.5 | 3 |
| 5/8 | Donors . . . . . | 16 | 75-C | 0.1 | 3.5 | 5.2 | 1.6 | 0.9 | 0.4 | 4 |
| 3/5 | Patients C . . . . . | 18 | 76-L | 0.1 | 3.6 | 3.2 | 1.9 | 0.3 | 0.1 | 3 |
| 1/21 | Donors . . . . . | 19.5 | 76-L | 0.1 | 0.2 | 0.7 | 0.5 | 0.5 | 0.1 | 2 |
| 2/20 | Donors . . . . . | 18.5 | 76-L | 0.1 | 0.3 | 0.4 | 0.5 | 0.4 | 0 | 2 |
| 5/8 | Donors . . . . . | 16 | 76-L | 0.1 | 1.9 | 4 | 1.4 | 0 | 0 | 3 |
| 3/5 | Patients C . . . . . | 18 | 77-D | 0.1 | 2.9 | 2.9 | 1.2 | 0.9 | 0.3 | 3 |
| 1/21 | Donors . . . . . | 19.5 | 77-D | 0.1 | 1.1 | 3.3 | 2.8 | 0.5 | 0.3 | 3 |
| 2/20 | Donors . . . . . | 18.5 | 77-D | 0.1 | 1.3 | 2.5 | 2.0 | 2.0 | 0.8 | 3 |
| 5/8 | Donors . . . . . | 16 | 77-D | 0.1 | 0.9 | 1.0 | 0.9 | 0.5 | 0.2 | 3 |

Note. Sera: C) against serum γ-globulins of patients with cirrhosis of the liver; L) of patients with leprosy; D) of donors; 3) X-component formed an indistinct arc of precipitation.

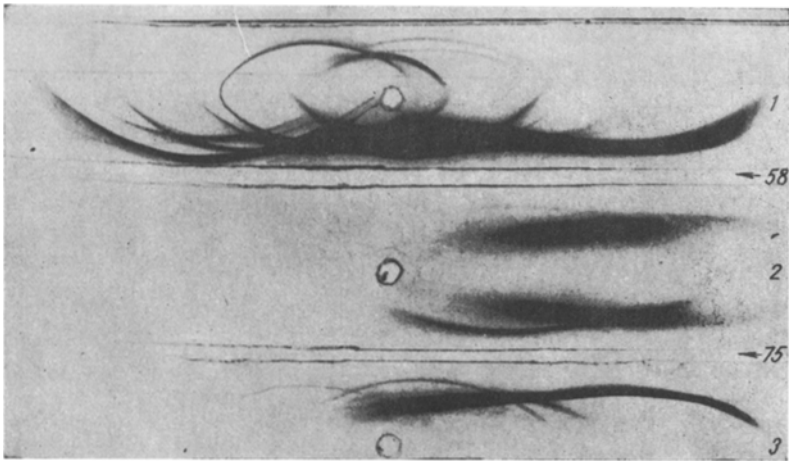


Fig. 1. Immunoelectrophoresis of γ-globulin after spontaneous breakdown and characteristics of antiserum to this protein. 1 and 3) Human blood serum; 2) serum γ-globulin 14 months after being taken; sera: 58) against whole human serum, 75) against γ-globulin.

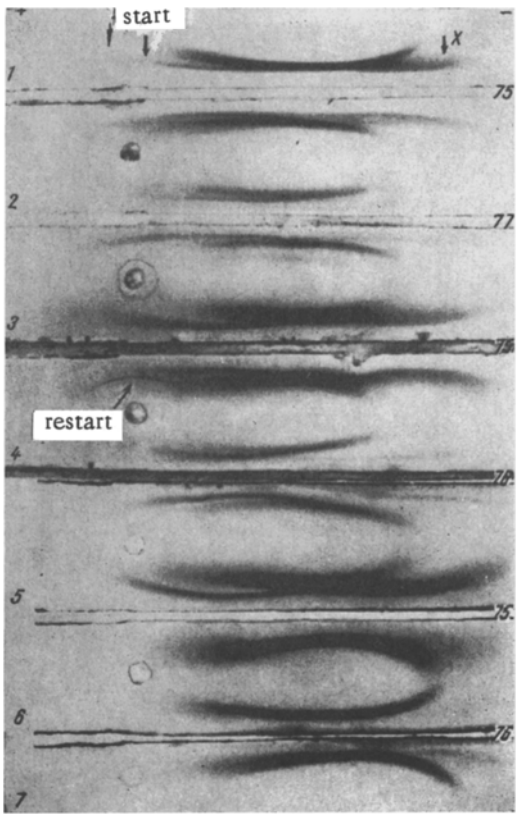


Fig. 2. γ-Globulins and their components detected by immunoelectrophoresis during treatment with different antisera (experiment on September 6, 1962). Dates of obtaining γ-globulins: 1) 1/21/1961; 2) 2/20; 3) 3/5; 4) 5/8; 5) 3/3; 6) 5/1; 7) 5/3. The numbers are the serial numbers of the antisera.

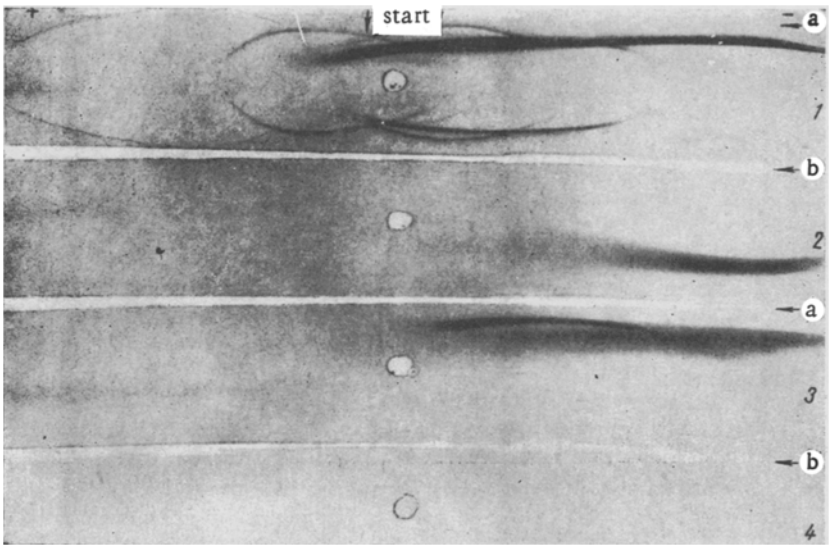


Fig. 3. Reaction of antigammaglobulin serum (75-C) before (a) and after (b) specific adsorption with freshly prepared γ-globulin (experiment on November 2, 1962). 1) Human blood serum; γ-globulins and dates of obtaining; 2) 10/24/1962; 3) 3/3/1961; 4) 3/5/1961.

## EXPERIMENTAL METHOD

Preparation of γ-Globulin. Pooled serum from several donors, and individual and pooled sera from patients with severe hypergammaglobulinemia (cirrhosis of the liver, leprosy), were fractionated by electrophoresis on a starch block in an apparatus of the Kunkel type [2]. The purity and homogeneity of the resulting preparations were tested by immunoelectrophoresis in jelly, using rabbit antiserum against whole human blood serum. This antiserum contained precipitins in a concentration of about 20 mg% ml and was capable of detecting as many as 18 antigenic components in whole human serum, whereas it reacted with the preparations which we obtained to give one precipitation line, characteristic of γ-globulins.

The γ-globulins were eluted from the starch into veronal-medinal buffer (pH 8.6, ionic strength 0.1) and kept in the same solution at a temperature of about 4° for a long period of time (from a few months to 3 years).

Preparation of Antisera. Rabbits were immunized with solutions of the γ-globulins in accordance with the scheme described previously [3]. Antisera obtained after the first immunization were used in the experiments, and contained precipitating antibodies against γ-globulins in a concentration of not less than 3 mg/ml. Two batches of antisera against freshly prepared γ-globulins of donors (No. 74) and patients with leprosy (No. 76), forming only one precipitation line during electrophoresis with whole human serum, were used in the investigation. Two other batches of antisera against γ-globulins from donors (No. 77) and from patients with cirrhosis or the liver (No. 75), stored for a long time, not only reacted with γ-globulins, but also formed clear precipitation lines in the zones of the β- and α-globulins and a weak are in the zone of the albumins (Fig. 1). Antisera against the serum proteins of healthy donors and patients with leprosy were used as controls.

Immunochemical Analysis. Precipitation curves were plotted for each preparation of γ-globulin with the resulting antisera by Heidelberger's method, after which the precipitation reaction was repeated every 3 months with the addition of definite amounts of antigen. The protein concentration in the solutions and precipitate was determined by the method of Lowry and co-workers [11].

Immunoelectrophoresis was performed in the chamber of a type ÉFA-1 apparatus, specially adapted for micro- and macroanalysis. The experimental conditions were : voltage 200 V, current 10 mA when the chamber was fully charged, duration 6 h. The buffer solutions for the electrodes and agar were prepared from the formula of Laurell and co-workers [10]. Observations were made on 20 preparations of human serum γ-globulins obtained at different times from different sera, but under the same experimental conditions.

## EXPERIMENTAL RESULTS

The rabbit serum against γ-globulin clearly revealed its heterogeneity and also formed precipitation lines with whole serum in the zones of the albumin and α- and β-globulins (see Fig. 1). The same antiserum formed one precipitation line, characteristic of γ-globulins, with freshly prepared γ-globulins whereas with γ-globulins stored for between 6 and 8 months it revealed heterogeneity (Fig. 2). It is clear from Figs. 1 and 2 that, irrespective of the period of storage of the spontaneously disintegrating γ-globulins, their three-component structure was distinctly visible. A component with greater mobility and another with smaller mobility than the principal peak could be seen. Skvaril [14] refers to these components as the Y- and X-fragments of γ-globulin respectively and considers that the slow X-component is separated from the principal peak after formation of the Y-component.

According to our findings the Y-component began to separate from the principal peak of the γ-globulin in the 4th month of storage of the preparation and attained full development at the end of the 6th-7th month. At roughly the same times another arc of precipitation was formed, by the X-component. The latter was most clearly detected by means of antisera obtained by immunization with γ-globulins after long storage (see Fig. 2). By using these antisera it was possible to detect the formation in two preparations, at the 10th month of storage, of a fourth arc of precipitation belonging most probably to an unknown component possessing slightly greater mobility than the Y-component (we call it conventionally the pre-Y-component).

To study the specificity of the antigenic determinants of the individual components of the spontaneously disintegrating γ-globulin we used the method of specific adsorption or the method of exhaustion of antisera. For instance, the antisera obtained as a result of immunization of rabbits with three-component γ-globulins were able to detect not only γ-globulins in the whole serum, but also $\beta_2$- and β-globulins, not less than two fractions in the zone of the α-globulins, and one in the zone of the albumins. These antisera, exhausted both with freshly prepared and with stored γ-globulins. continued to form arcs of precipitation in all the above-mentioned zones apart from the precipitation line characteristic of the γ-globulins (Fig. 3), and also revealed pre-Y-components in the four-component preparations of γ-globulin. The same antisera, when specifically adsorbed with four-component γ-globulins, lost their ability to react both with whole serum and with all the preparations of the γ-globulins.

Consequently, the three components of the γ-globulin molecule most frequently formed as a result of the spontaneous breakdown of this protein had identical antigenic determinants with normal γ-globulin. In individual cases during the formation of a pre-Y-component new determinants began to be formed, among which some were identical with the antigenic groups of certain fractions of the β- and α-globulins. If it is remembered that the antisera forming arcs of precipitation during electrophoresis in the zones of the α- and β-globulins were prepared by immunizing rabbits with three-component γ-globulins, the following hypothesis may be submitted. During immunization dissociation of the Y-component takes place in the rabbit, with the separation of a pre-Y-component, as a result of which latent antigenic determinants are liberated, some of which are identical with the antigenic groups of the α- and β-globulins. This may possibly account for the difficulty in obtained monospecific antisera during immunization even with homogeneous preparations of γ-globulins. In any case the cross reactions obtained in the course of our earlier experiments on active anaphylaxis [2] demonstrated that antigenic determinants identical with α- and β-globulins are present in horse serum. These findings are in agreement with the data recently obtained by A. Ya. Kul'berg and co-workers [9], who experimented with rabbit γ-globulins.

Comparison of the precipitation curves obtained as a result of the reaction of the antisera on the addition of equal amounts of different γ-globulins shows that certain antisera are capable of detecting additional features of the antigenic structure of the protein.

It is clear from the table that antiserum (75-C) to the γ-globulins isolated from the serum of the patients with cirrhosis of the liver formed a much larger precipitate with this preparation at all points of the precipitation curve than with the γ-globulins of the donors stored for the same periods. It might have been possible in this case to regard the quantitative precipitation reaction as specific, had not analogous differences been found in the reaction between these preparations and antiserum to the γ-globulins of the patients with leprosy. Nevertheless, it appears probable that efforts in this direction might be interesting from the point of view of investigation of the immunological properties of normal and "pathological" γ-globulins after spontaneous breakdown. Accordingly, attention was directed to the fact that the γ-globulins isolated from sera of patients with leprosy acquired their three-component structure slightly earlier than normal γ-globulins if all other experimental conditions were identical. Admittedly, the existing facts are inadequate to allow more definite conclusions to be drawn in this matter.

SUMMARY

Gamma-globulins were isolated from human blood serum on a starch block in an apparatus of the Kunkel type. The proteins were kept in a veronal-medinal buffer (pH 8.6, ionic potential – 0.1) at a temperature of about +4°C for a period ranging from a few months to 3 years. Gamma-globulin homogenicity was tested periodically with the aid of immunoelectrophoresis; precipitation properties were tested by the Heidelberger quantitative method.

Gamma-globulins kept for a long time in solution as a rule disintegrate, with the formation of three antigenic components similar in their immunological properties to normal gamma-globulin. In spontaneous disintegration of the gamma globulin the separation of a fourth i.e., of the pre-Y-component, may occur.

A suggestion is made that, apart from the gamma-globulin determinants, the pre-Y-component contains latent groups, similar to antigenic determinants $\beta_2$ – A, $\beta_1$, to some gamma-globulin fractions, and possibly even to the antigenic albumin determinants.

LITERATURE CITED

1. Yu. S. Tatarinov, Byull. éksper. biol., 10, 97 (1960).
2. Yu. S. Tatarinov, Biokhimiya, 2, 306 (1960).
3. R. Ausutin and B. Hayward, Nature (1960), 187, p. 129.
4. R. Ausutin and B. Hayward, Immunology (1961), 4, p. 450.
5. V. Balazs, R. Backhausz, and M. Fröhlich, Magy, Tud. Akad. Biol. orv. Tud. Osztal. Közl. (1960), 11, 441.
6. P. Grabar, Biokhimiya (1957), 1-2, p. 49.
7. L. A. Hanson and B. G. Johansson, Nature (1960), 187, p. 599.
8. T. Ishizaka, D. Campbell, and K. Ishizaka, Proc. Soc. exp. Biol. (N. Y.) (1960), 103, p. 5.
9. A. Kulberg, I. Tarkhanova, and N. Khramkova, Folia biol. (Prague) (1960), 7, 3, p. 213.
10. C. B. Laurell, S. Laurell, and N. Skoog, Clin. Chem. (1956), 2, p. 99.
11. O. H. Lowry, N. J. Rosebrough, A. L. Farr et al., J. biol. Chem. (1951), 193, p. 265.
12. R. R. Porter, Biochem. J. (1957), 66, p. 677.
13. E. M. Press and R. R. Porter, Biochem. J. (1962), 83, p. 172.
14. F. Skvaril, Nature (1960), 185, p. 475.

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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.**

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