ANTIANAPHYLACTIC ACTIVITY OF FRACTIONS OBTAINED BY FRACTIONATION OF γ-GLOBULIN THROUGH SEPHADEX G-200 GEL

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Of five fractions isolated by fraction of γ-globulin on Sephadex G-200 gel, fractions III and IV, containing the greater part of the γG-immunoglobulin and its fragments, possessed the least inhibitory activity with respect to passive cutaneous anaphylaxis.

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Facts relating to the antiallergic effect of γ-globulin [1, 2, 5, 6] are of theoretical and practical interest. However, much is unexplained in mechanism of this phenomenon. In particular, the antiallergic properties of the serum protein fractions are not clear.

In the present investigation the antiallergic activity of protein fractions of a commercial γ-globulin preparation, obtained by fractionation through Sephadex G-200 gel, was investigated.

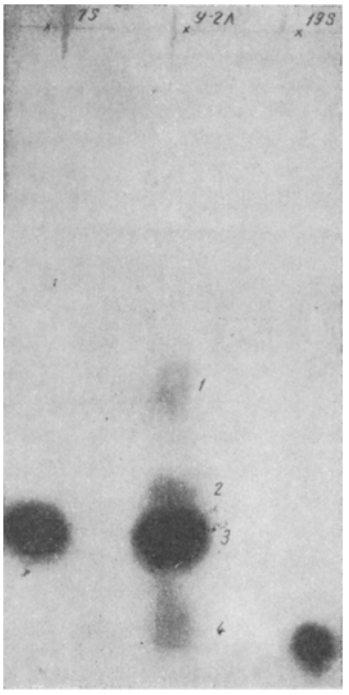

Fig. 1. Thin-layer chromatography of γ-globulin of batch No. 207 fractionated on Sephadex G-200 gel. 1) albumin; 2) fragments of γG-immunoglobulin; 3) 7S-immunoglobulins; 4) 19S-immunoglobulins.

EXPERIMENTAL METHOD

A production batch of γ-globulin manufactured at the I. I. Mechnikov Research Institute of Vaccines and Sera, Ufa, from human blood serum obtained during a therapeutic abortion operation, was used for the investigation.

Fractionation through Sephadex G-200 gel was carried out on a column measuring 30 × 800 mm. The γ-globulin preparation was diluted 1 : 2 with boro-borate buffer (pH 7.2, 0.01 M) and dialyzed in the cold against the original buffer solution. A 5% solution of γ-globulin was applied to the column in a volume of 6 ml. Protein was eluted by the above-mentioned buffer solution at the rate of 12-18 ml/h. The eluate was collected in samples of 1 ml, in which the protein content was determined by Lowry's method, and a chromatographic curve was plotted. The total eluate was divided into five fractions.

After concentration and dialysis of the fractions against physiological saline their protein composition was studied by Grabar's method of immunoelectrophoresis and by thin-layer chromatography as described previously [3].

The phenomenon of passive cutaneous anaphylaxis (PCA) was produced in noninbred guinea pigs with white hair by means of a rabbit antiserum against bovine serum albumin and specific antigen by the method described previously [2].

I. I. Mechnikov Research Institute of Vaccines and Sera, Ufa. (Presented by Academician of the Academy of Medical Sciences of the USSR N. N. Zhukov-Verezhnikov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 70, No. 7, pp. 72-73, July, 1970. Original article submitted October 31, 1969.

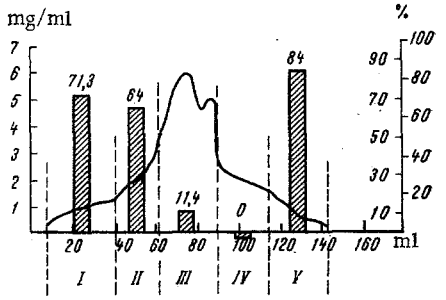

Fig. 2. Inhibitory activity relative to PCA of fractions obtained by fractionation of γ-globulin of batch No. 207 on Sephadex G-200 gel. I, II, III, IV, V: serial numbers of fractions. Shaded columns represent intensity of PCA (in % of control). Ordinate, on left – protein concentration (in mg/ml); on right – intensity of PCA (in %); abscissa, volume of eluate (in ml).

EXPERIMENTAL RESULTS

Heterogeneity of the protein composition of the γ-globulin preparation was discovered by thin-layer chromatography using Sephadex G-200 (Fig. 1).

The experiments were carried out on ten guinea pigs. The antigen with physiological saline (control) and with the fractions of γ-globulin previously obtained were injected intradermally into the animals. These fractions were used as a mixture with antiserum in a dose of 1.2 mg/ml. The intensity of the phenomenon in the experimental series was expressed as a percentage of its intensity in the control. These experiments showed that the greater part of the antianaphylactic activity is concentrated in fractions III and IV; fractions I, II, and V were much less active (Fig. 2). Fraction IV possessed the greatest activity, and besides γG-immunoglobulin it also contained as impurities fragments of spontaneous degradation of this immunoglobulin. The highly active fraction III contained the greater part of the γG-immunoglobulin. Of the fractions with low activity, fraction I contained γM-immunoglobulin, and fraction II contained γG-immunoglobulin and slight traces of γA-immunoglobulin. Fraction V, possessing the lowest activity, contained albumins and globulins not belonging to the "γ" system.

The facts obtained thus show that the greater part of the antianaphylactic activity of γ-globulin preparations is associated with γG-immunoglobulin and its fragments appearing through spontaneous degradation [4, 7]. γM-immunoglobulin possesses only slight antianaphylactic activity. So far as the weak inhibitory function of fraction II, containing γG-immunoglobulin and only slight traces of γA-immunoglobulin, is concerned, this fact may perhaps be explained by heterogeneity of the γG-immunoglobulin.

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