ON THE IMMUNOLOGIC CHARACTERISTICS OF SERUM PROTEINS

Part 1. Induction of Anaphylaxis by Isolated Fractions of Blood Serum Proteins

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At present, one may consider as established that individual electrophoretically pure fractions of serum proteins are immunologically heterogeneous [2, 5, 7, 8, 9].

Data are also available dealing with the possibility of specific cross reactions between albumins and globulins [3].

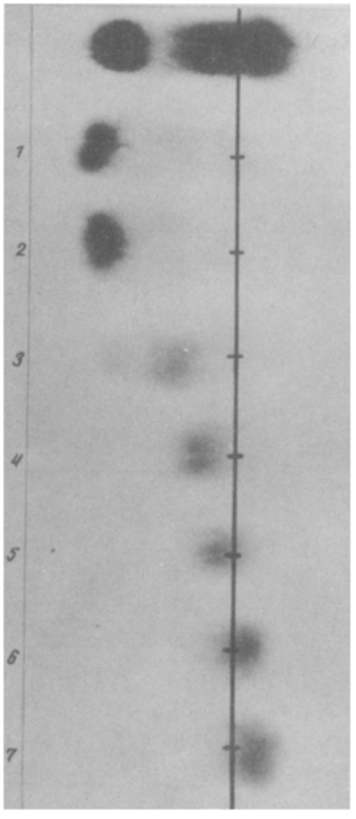

Figure. Electrophoresis of isolated protein fractions separated on starch block (paper B 12 x 40 cm, 450 v, 2.5 ma, 4 hr). 1,2) Albumins; 3) α_1-; 4) α_1-; 5) β-; 6) γ_1-; 7) γ_2-globulins; above - normal horse serum.

In this connection, it appeared interesting to clarify whether it is possible to differentiate anaphylactogenic properties of different isolated fractions of serum proteins.

The available pertinent data from literature are contradictory. Experimental results reported by Svingendow and his co-workers [4] were not substantiated by experiments of N. A. Gorbunova and T. M. Tsipin [1]. The former authors believe that it is not essential to introduce different fractions of serum proteins (albumins and globulins) as sensitizing and challenging injections for induction of anaphylactic shock in guinea pigs.

To resolve this question we utilized, as a model of anaphylactic reaction, anaphylactic shock in guinea pigs.

METHODS

Fractionation of serum. Separation of normal horse serum proteins was carried out in an apparatus similar to that described by Kunkel, utilizing a starch block (1x13 x26 cm) in median-veronal buffer (pH 8.6, ionic strength 0.1) for 48 hr under 17.3 v/ cm potential gradient and 10 ma current. Visual observation of migration of albumins and globulins in the course of electrophoresis has been insured by addition of a small quantity of Evans' blue (T-1824) to the serum.

Isolation of protein fractions. A part of the starch block containing proteins was cut into individual cross sections 1 cm wide. Proteins were extracted from starch with the help of the median-veronal buffer. The amount of protein in each sample was determined using Lowry's method [6] and the distribution of proteins in the starch block was plotted graphically. Control of purity of protein fractions isolated from starch was carried out with the help of micropaper electrophoresis. The protein solutions obtained wereanalyzed in cold against physiological saline for 30 hr.

Sensitization and shock. Experiments were carried out using male and female guinea pigs weighing 200 to 450 g. One or two sensitizing injections were given sub-

Induction of Active Anaphylaxis in Guinea Pigs by Purified Fractions of Serum Proteins

Animal number	Wt. of animal (in g)	Sensitizing injection			Shocking injection				Nature of anaphylactic reaction
		date (1959)	protein fraction	amount of protein (in mg)	day of reinoculation of antigen	protein fraction	amount of protein (in mg)	volume of solution (in ml)	
73	435	Jan. 10, 12	A	1.5	20	A	3.5	1.0	++++
74	230	Jan. 10, 12	A	1.5	20	α_1-g	3.5	1.2	+
					21	A	3.5	0.1	++++
70	324	Jan. 10, 12	A	1.5	20	α_2-g	3.0	1.1	—
					21	A	3.5	0.1	++++
125	240	Nov. 3	A	1.0	21	γ-g	4.0	1.0	—
					21	A	3.2	0.1	++++
119	290	Nov. 3	α_1-g	1.0	21	A	3.5	0.4	++++
120	360	Nov. 3	α_2-g	1.0	21	A	3.5	0.4	++++
91	350	June 16	α_2-g	1.0	21	α_1-g	2.5	1.0	++++
90	350	June 16	α_2-g	1.0	21	β-g	1.2	0.8	++++
92	440	June 16	α_2-g	1.0	21	γ-g	1.5	0.5	++++
93	415	June 16	β-g	1.0	21	A	7.0	1.0	—
122	410	Nov. 3	β-g	1.0	21	A	4.0	0.5	—
					21	γ-g	4.0	1.0	++++
126	225	Dec. 4	γ_1-g	2.0	22	A	6.8	0.9	—
					22	α_1-g	2.0	1.0	++++
128	305	Dec. 4	γ_2-g	1.5	22	A	5.2	0.7	—
					22	α_2-g	2.8	0.9	+++
99	420	June 16	γ_2-g	1.0	21	β-g	2.0	1.0	++++
130	285	Dec. 4		0.5	22	γ_1-g	2.7	0.9	++++

Legend: A—albumins; g—globulins; ++++ severe anaphylactic shock leading to death in 2-5 minutes; +++ prolonged anaphylactic shock leading to death a few hours after injection of antigen; + anaphylactic reaction in the form of scratching, coughing, shaking, and with rapid passing of these symptoms; — without symptoms characteristic of anaphylactic shock.

cutaneously using a solution of electrophoretically pure protein fraction (from 0.2 to 5 mg protein).

Shocking dose (1 to 7 mg protein) was administered intracardially on the 20-22nd day after the sensitizing dose. Forty-five guinea pigs were used in these experiments.

RESULTS

As can be seen from the table, all the protein fractions of normal horse serum were equally anaphylactogenic when tested on guinea pigs, and a pronounced anaphylactic shock was observed following administration of shocking dose of the corresponding antigen. The results obtained confirm the known position about the specificity of anaphylactic phenomena being within the boundry of one and the same type of serum proteins. However, using albumins for sensitizing dose and globulin (except α_1- globulins) for shocking dose makes it impossible to bring about a general anaphylactic reaction. Anaphylactic shock was also absent when β-and γ- globulins were used for sensitization and albumins for shocking dose. Cross anaphylactic reactions occasionally observed when α- globulins were used for sensitization and albumins for shocking dose are apparently conditioned in that these globulin fractions contain different amounts of albumin impurities. As can be seen on the figure, α_1- globulins isolated from a starch block have noticeable amounts of proteins with electrophoretic mobility of albumins. However, it is not possible to uncover proteins with electrophoretic mobility of albumins in paper electrophoresis of α_2- globulins. However, if we consider that active sensitization is possible by introduction of 0.001 mg of protein, then the cross anaphylactic reactions may be also carried out because of insufficient homogeneity of α_2-globulins, although this is not made apparent upon paper electrophoresis.

When the isolated globulin fractions are used for sensitization, anaphylaxis can be brought about by using any globulin fraction as shocking dose. In addition specific desensitization may be accomplished using any of these fractions. However, guinea pigs sensitized with globulins, and which were shocked by injections of albumins, remain sensitive to subsequent injection of globulins. These data point to great similarity in anaphylactogenic properties of isolated fractions of serum globulins separated by means of starch block electrophoresis.

Results obtained by us are indicative of anaphylactogenic specificity of electrophoretically pure fractions of albumins in comparison with β-and γ-globulins.

SUMMARY

Preparatory isolation of electrophoretically pure protein fractions from normal horse serum was carried out with the help of apparatus of Kunkel type in a starch block (1x13x26 cm) with 450 v, 10 ma during 48 hours.

Experiments on guinea pigs with production of active anaphylaxis have shown that albumins and globulins possess different anaphylactogenic specificity, while individual fractions of globulins possess identical immunogenic properties and are capable of giving cross anaphylactic reactions.

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* Original Russian pagination. See C. B. translation.