PROBLEM OF HAPTEN COMPETITION IN ARTIFICIAL ANTIGENS

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Vaccines and toxoids employed in medicine represent mixtures of antigens of complex structure. Often one makes use of combinations of various toxoids and vaccines. In this connection the question of the competing influence of the antigens in immunization assumes great importance.

This question has been studied by means of immunization of animals with a mixture of nitro- and azo-albumins. L. I. Ilyina and A. P. Konikov [1] used as antigens nitro-albumins, anilinazo-albumins, sulfanilazo-albumins and atoxilazo-albumins. V. I. Vashkov [2] used as hapten, ortho, meta and paraminobenzoic acids and also meta- and para-aminobenzenesulfonic acids. These investigations established that combined immunization with synthetic antigens, each of which possesses individual specificity, never leads to complete suppression of the individual specificity. In relation to each of the antigens it is seen that nitro-albumins in a combination with azo-albumins produce just as active antibodies as do nitro-albumins alone in individual immunization [1].

Analinazo-albumins in combination with the other antigens in the majority of cases display a clear fall in antigenic capacity; the activity of atoxilazo-albumins is suppressed in immunization conjointly with other antigens; the antigenic function of sulfanilazo-albumins is reduced in combination with other antigens. V. I. Vashkov, by immunizing rabbits with albumins combined with ortho, meta and paraminobenzoic acids and meta and para-aminosulphonic acids observed a higher titer of precipitins on immunization by a mixture of acids with a radical at the meta position than with immunization by ortho and para acids.

EXPERIMENTAL METHODS

We investigated the competition of two haptens in one molecule of albumin. Mixtures of artificial antigens having in the molecule one hapten served as control. By the azotization method one and two different haptens were introduced in the molecule of the globulins. We prepared sulfanilazo-globulins, antranilazo-globulins, nitroanilinazo globulins, anilinazo globulins (monoazo globulin derivatives) sulfanilantranilazo-globulins, sulfanilnitroanilinazo-globulins, sulfanilanilinazo-globulins, anilinnitroanilinazo-globulins (diazo globulin derivatives).

For the purpose of immunization azoglobulins prepared from the serum globulins of a horse were used and as test object we used azo globulins prepared from the serum globulins of human retroplacental blood.

The globulins were obtained by precipitation of the blood serum with an equal volume of saturated solution of ammonium sulfate with subsequent dialysis against running water. The globulin solution freed from the ammonium sulfate was cooled to O°C and decanted by agitation into five times its volume of acetone at a temperature of 5°C. The globulin residue was quickly removed by filtration and freed from acetone by cold ether. The white, friable powder obtained was dried and stored in sealed ampules.

In order to obtain diazo compounds 0.5 M solution of the aromatic amine in a 1.5 M solution of hydrochloric acid (3 mol of hydrochloric acid to 1 mol of aromatic amine) was prepared. To the solution cooled to O°C with shaking in the electro-mixer 0.1 M sodium nitrate solution was gradually added.

The end point of diazo titration was determined by means of iodized starch paper.

In order to obtain a mixture of the diazo compounds 0.5 M solution of the equimolecular relations corresponding to the aromatic amines was prepared.

The azoglobulins were obtained by means of gradual addition with shaking of the diazonium salts cooled to O°C (mixture of diazonium salts) to a 2% globulin solution at the same temperature in 0.1 M alkaline solution.

The end point of the reaction was determined by means of α naphtol in alkaline medium which with a surplus of diazonium salt gave a vivid red color. The reaction was considered complete if the mixture was colored 15 minutes after addition of the diazonium salt. A dark red solution of azoglobulin, to complete the reaction, was left to stand for another 20-30 minutes at room temperature and was then acidified with 1.0 M hydrochloric acid until a blue color was produced with congo red paper. Large flocules of azo-albumin then formed, which were removed by means of centrifugation. The residue was washed with water until colorless washings were obtained, dissolved in 0.1 M alkaline solution, chilled to O°C and decanted with shaking into five times its volume of acetone at 5°C. The precepetate forming was rapidly removed by filtration and washed free of the acetone with cold ether. The powdered azo-albumin obtained was dried and stored in a sealed ampule or in a well closed test tube. Rabbits were immunized with a mixture of equal amounts by weight of monoazo-globulin derivatives and with diazo-globulin derivatives.

We investigated the following mixtures of monoazo globulin derivatives: sulfanilazo-globulin and antranilazo-globulin, sulfanilazo-globulin and nitroanilinazo-globulin, sulfanilazo-globulin and anilinazo-globulin, anilinazo-globulin and nitroanilinazo-globulin. Two rabbits were immunized with each mixture and three rabbits with each diazoglobulin derivative. The rabbits were immunized for 6 days by daily intravenous injections of 1 ml of 1% azo-globulin solution; after interruption for a week the series of injections was repeated. On the tenth day after the last injection the rabbits were deprived of blood. The serum obtained was stored in an ice box in sterile test tubes. Determination of the precipitins in the sera was performed by the method of ring precipitation.

The test antigens were standardized according to weight; we used as the basic weight an 8% solution of antigen from which were prepared attenuations 1:10, 1:160, 1:2560, 1:10, 240, 1:40, 960, 1:123, 840. To 0.5 ml of immune serum a 1 ml layer of antigen of the corresponding attenuation was added. After 2 hour incubation at 37°C the result of the reaction was seen. The strength of the anti-serum was established by means of determining the greatest attenuation of the antigen giving ring precipitation with the anti-serum.

EXPERIMENTAL RESULTS

The results of the experiments are presented in the table.

It is clear from the table that the sera obtained upon immunization with the mixture of monoazo-globulins and diazo-globulins were of uniform strength. The sulfanil-haptens and the antranil-haptens suppressed to an insignificant degree the nitroanilin-haptens and to a greater extent the anilin-haptens. In its turn the nitroanilin-hapten suppressed the anilin-hapten.

No complete suppression of the antigenic function took place. Cross reactions between azo-globulins, containing nitroanilin and anilin-haptens were observed.

The mixture of the monoazo- and diazo- derivatives in equal parts by weight used as test antigen did not exert any appreciable influence on the result of ring precipitation - they reacted as antigens at an attenuation double that of the monoazo-derivatives.

Thus, the mixture of antigens of the monoazo- and diazo- derivatives gave a uniform effect both in immunization and in the ring precipitation reaction in the capacity of a test antigen.

As a result of the experiments conducted it was established that:

1) The antigens maintain their specificity upon immunization of the animals both with the mixtures of monoazo-globulins and of diazo-globulins;

2) upon immunization with the mixture of the monoazo-globulins and diazo-globulins precipitins are formed, the titers of which do not essentially differ from one another;

Ring Precipitation Reaction With Rabbits Sera Immunized With Diazo-Globulins and with a Mixture of Monoazo-Globulins

	Immunizing antigen	No. of rabbit	Maximum attenuation of test antigen of azo globulin of human serum											
			antranil-azo globulin	sulfanil-azo globulin	nitroanil-inazo globulin	anilinazo globulin	antranil- + sulfanil azo globulin	sulfanil + + nitro-anilinazo globulin	sulfanil anilinazo globulin	nitro-anilin-anilinazo globulin	antranil-sulfani-nilazo globulin	Sulfanil-nitro-anilin-azo globulin	sulfanil-anilinazo globulin	nitroanilin-anilinazo globulin
1	Antranil + sulfanilazo globulin	1	2,560	10,240	—	—	640	640	640	—	2,560	160	640	—
		2	10,240	10,240	—	—	2,560	640	2,560	—	2,560	640	640	—
2	Antranil-sulfanilazo globulin	3	40,960	10,240	—	—	10,240	2,560	2,560	—	10,240	2,560	2,560	—
		4	10,240	40,960	—	—	10,240	2,560	10,240	—	10,240	2,560	10,240	—
		5	10,240	10,240	—	—	2,560	640	2,560	—	2,560	640	640	—
3	Sulfanil + nitroanilinazo globulin	6	—	40,960	2,560	40	640	10,240	2,560	640	640	2,560	640	160
		7	—	40,960	10,240	40	2,560	10,240	10,240	2,560	2,560	2,560	2,560	160
4	Sulfanil-nitroanilinazo globulin	8	—	10,240	2,560	40	640	10,240	640	640	2,560	2,560	2,560	640
		9	—	10,240	10,240	160	2,560	10,240	10,240	2,560	2,560	10,240	2,560	2,560
		10	—	2,560	640	40	640	640	640	640	640	2,560	2,560	640
5	Sulfanil + anilinazo globulin	11	—	40,960	160	640	2,560	2,560	2,560	160	640	640	2,560	160
		12	—	2,560	40	160	640	640	2,560	160	160	640	640	40
6	Sulfanil-anilinazo globulin	13	—	10,240	40	160	640	2,560	2,560	160	640	2,560	2,560	640
		14	—	640	10	40	160	160	160	160	160	160	160	
		15	—	10,240	40	640	640	640	640	160	640	640	640	160
7	Nitroanilin + anilinazo globulin	16	—	—	2,560	640	—	160	160	640	—	640	160	640
		17	—	—	2,560	2,560	—	160	160	160	—	160	160	640
8	Nitroanilin-anilinazo globulin	18	—	—	640	160	—	160	40	640	—	160	40	640
		19	—	—	2,560	640	—	640	160	2,560	—	160	40	2,560
		20	—	—	2,560	160	—	160	40	640	—	40	40	640
9	Control - normal horse serum	—	—	—	—	—	—	—	—	—	—	—	—	—

3) there exists an identity between the competition of the haptens in the monoazo-globulins upon their joint introduction with competition of the haptens in the diazo-compounds;

4) the mixture of monoazo-globulins and diazo-globulins, taken as test antigens, did not exert an important influence on the results of ring precipitation;

5) synthesis of an artificial antigen with dual specificity appears possible.

LITERATURE CITED

[1] Ilyina, L. I., and Konikov, A. P., Zhur. Mikrobiol., Epidemiol. i Immuniol. 1941, 4, 83-90.

[2] Vashkov, V. I., Zhur. Mikrobiol., Epidemiol. i Immuniol. 1941, 4, 90-93.