CHANGES IN THE ANTIGENIC PROPERTIES OF THE BLOOD SERUM AFTER PARENTERAL INJECTION OF NONPROTEIN SUBSTANCES INTO AN ANIMAL

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We have shown [3] by the method of immunization, sensitization, desensitization, and anaphylaxis, that the antigenic function is a property of substances of protein nature only. The formation of antibodies to substances of nonprotein nature is explained by the formation of complexes by the substances injected and the animal's own proteins, so that the proteins are denatured and converted into foreign protein [1].

Denaturation changes in the serum proteins of horses hyperimmunized with antitoxins are shown, in particular, by the fact that diphtheria, tetanus, and botulinus antisera are extremely resistant to heat [2, 4]. Hypotheses concerning the changes in the antigenic properties of the proteins of the body by the action of injected compounds have not yet, however, received experimental confirmation.

In the present investigation our aim was to bring to light denaturation changes in the serum proteins and in their antigenic properties.

METHOD AND RESULTS

In order to dectect denaturation changes in the serum proteins after injection of various substances into animals, we used F. S. Okolov's method [4]. Rabbits were immunized with 1% solutions of horse serum sulfanylazoglobulin, sulfanylazostarch, sulfanylazosalicylic acid and a mixture of this with serum, starch solution, a 5% solution of sulfanylazosalicylic acid, and normal horse serum (control). On the day of the last injection, the animals' serum was tested for its thermal stability, and on the 8th day its antibody content was determined by the precipitation method. The thermal stability of the serum proteins was determined in a volume of 1 ml, at 61-62°. Heating was contined until the serum was completely gelatinized.

The results of the experiments are shown in Table 1.

It will be seen from Table 1 that solutions of starch and sulfanylazosalicyclic acid, when given by the usual methods of immunization, did not bring about the appearance of antibodies and did not affect the thermal stability of the serum proteins. A 5% solution of sulfanylazosalicylic acid sharply increased the thermal clotting time of the sera and led to the formation of precipitins (rabbits nos. 18, 19, and 20). An analogous phenomenon, although less pronounced, was observed after injecting the animals with sulfanylazoglobulin, sulfanylazostarch and a mixture of sulfanylazosalicylic acid with protein. In a control experiment, no changes were observed in the thermal clotting time.

This series of experiments thus demonstrated a link between the formation of precipitins to azo-compounds and the denaturation changes in the serum proteins of animals. No quantitative relationship could be found between the thermal clotting time and the antibody titer.

In the next series of experiments, wer tested the changes in the antigenic properties of the body proteins under the influence of sulfanylazostarch and sulfanylazosalicylic acid. In this case we took into consideration the fact that substances not possessing antigenic properties may desensitize animals for several hours [3, 5, 6], but true antigens for a much longer period (2-3 weeks). With regard to the substances under test, we found that [3] sulfanylazostarch desensitized guinea pigs for periods of up to 24 hours, but sulfanylazosalicylic acid only for up to ½ hour; a mixture of solutions of sulfanylazosalicylic acid and protein possessed a prolonged desensitizing action. It could be concluded from these results that sulfanylazosalicylic acid and sulfanylazostarch had no inherent antigenic function, but the immunological specificity of the proteins was modified under their influence. These observations paved the way for experiments to determine the changes in the antigenic properties of the proteins in the animal body.

In order to modify the antigenic properties of the serum proteins of animals the preparations were injected intravenously into rabbits in a dose of 10 ml of a 4% solution, over a period of 2 days. On the second day, 4 hours after injection, the serum was tested for the presence of proteins with modified antigenic properties, by

TABLE 1 Antibody Titer and Thermal Clotting Time of Immune Sera

Immune serum	Rabbit No.	Dilution of test antigen—human sulfanylazoglobulin						Normal horse serum	Thermal clotting time (in minutes)	
		1:10	1:20	1:40	1:80	1:160	1:320		before immunization	after immunization
Against 1% starch solution	1	0	0	0	0	0	0	0	125	140
	2	0	0	0	0	0	0	0	105	130
Against 1% solution of sulfanylazosalicylic acid	3	0	0	0	0	0	0	0	135	140
	4	0	0	0	0	0	0	0	140	135
	5	0	0	0	0	0	0	0	100	115
Against 5% sulfanylazosalicylic acid solution	18	++	+	0	0	0	0	0	100	245
	19	+++	++	+	0	0	0	0	125	260
	20	++	0	0	0	0	0	0	120	200
Against 1% sulfanylazosalicylic acid solution in 1% horse globulin solution	15	+++	++	+	0	0	0	++++	125	165
	16	+++	++	0	0	0	0	++++	120	170
	17	+++	+++	++	0	0	0	++++	110	130
Against 1% horse sulfanylazoglobulin solution	9	++++	++++	++++	+++	+++	+	++++	100	205
	10	++++	++++	+++	++	+	0	++++	130	170
	11	++++	++++	++++	+++	+++	++	++++	95	165
Against 1% sulfanylazostarch solution	12	+++	++++	+++	++	0	0	0	120	215
	13	+++	+++	+	0	0	0	0	90	235
	14	++++	+++	++	+	0	0	0	120	190
Control—normal horse serum	21	0	0	0	0	0	0	++++	105	135
	22	0	0	0	0	0	0	++++	115	145
	23	0	0	0	0	0	0	++++	130	120

*Legend: ++++ flocculent precipitate, +++ slightly flocculent precipitate; ++ well-marked turbidity; + turbidity; 0 negative reaction.

TABLE 2 Desensitizing Power of Rabbit Sera Treated with Sulfanylazostarch and Sulfanylazosalicyclic Acid.

Desensitizing antigen	Guinea pig no.	Severity of anaphylactic shock	Prolongation of clotting time of the blood (in minutes)	Fall in temperature (in degrees)	Postmortem findings
Rabbit serum, treated with sulfanylazostarch	1	0	0	—0,1	—
	2	0	0(+)	0	—
	3	0	0	—0,3	—
	4	0	0	—0,1	—
	5	0	0	0	—
Rabbit serum, treated with sulfanylazosalicylic acid	6	1+	3	—1,1	—
	7	0	0	—0,1	—
	8	0	0	—0,3	—
	9	0	0	0	—
	10	0	0	0	—
Control 1—sulfanylazo-starch	11	++++	—	—	+
	12	++++	—	—	+
	13	++++	—	—	+
Control 2—sulfanylazo-salicylic acid	14	++++	—	—	+
	15	++++	—	—	+
	16	++++	—	—	+
Control 3—normal serum	17	++++	—	—	+
	18	++++	—	—	+
	19	++++	—	—	+

*Legend: 0) absence of reaction; +mild form of anaphylactic shock, ++++fatal anaphylactic shock.

densensitization of guinea pigs, preliminarily sensitized with horse serum sulfanylazoglobulin. The short interval of time elapsing between the beginning of injection of the test compounds and taking the blood prevented the antibodies from being concerned in the phenomenon of desensitization. Anaphylactic shock was indiced on the 4th day after desensitization by the intracordal injection of a 4% solution of human serum sulfanylazoglobulin, in a dose of 2 ml. The results of these experiments are shown in Table 2.

Rabbit sera treated with sulfanylazostarch and sulfanylazosalicylic acid thus cause prolonged desensitization of guinea pigs, previously sensitized to horse sulfanylazoglobulin. In all control experiments (desensitization with sulfanylazostarch, sulfanylazosalicylic acid, and normal rabbit serum), the animals died with manifestations of anaphylactic shock. These findings may be explained by the fact that the serum proteins of rabbits modify their antigenic properties under the influence of the injected azo-compounds.

It may thus be concluded from these experiments that nonprotein substances, injected into animals, denature the serum proteins, modify their immunological specificity and, in consequence of this, induce the formation of corresponding antibodies.

SUMMARY

The appearance of antibodies in response to the administration of nonprotein substances is accompanied by denaturalization of the serum proteins in the animals. The serum of rabbits injected with nonprotein substances acquires the ability to desensitize guinea pigs for prolonged periods of time. This leads to a conclusion that the introduction of nonprotein substances tends to denature the serum proteins, change their immunological specifity and, as a result of this, provoke the formation of corresponding antibodies.

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