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Use of formalin as an immunostimulant in animal immunization to produce specific serum precursors

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ABSTRACT
Immunostimulating property of formalin in the course of immunization of animals in obtaining specific precipitating high titre serum to determine the type of blood is experimentally established. The article presents the results of a comparative study of the adjuvant activity of 1%, 5% and 10% formalin solution in a mixture with human and animal blood serum. It is established that 10% formalin solution in the mixture with serum had the best adjuvant properties. Introduction of 10% formalin and blood serum solution into the mixture by animals contributed to obtaining immune serum precipitation suitable for use in forensic medicine.

Keywords: immunization, adjuvants, antigens, serum precipitating antigens.

INTRODUCTION
Search and introduction of new effective drugs with adjuvant properties for immunization of animals is an actual problem. Its solution will reduce the number of revaccinations, which will not only reduce the antigenic load on the body, but also significantly simplify and reduce the cost of the process of immunization of animals. Such adjuvants are subject to clear requirements: they should significantly increase the immunogenicity of vaccines and immune tension, and not possess toxicity and allergenicity (2, 3, 4).
Immunization is a method used exclusively in animals to obtain highly active therapeutic and protective or diagnostic serums from them. The immunization method is based on the general theoretical basis of immunobiology, which is confirmed by experiments that when artificially injected into an animal organism in some form of antigens - bacterial cultures, toxins, extracts and, in general, protein substances in it are formed after a certain period of time antibodies, which are concentrated mainly in the blood serum of the immunized animal (1, 5).
Nowadays, the widely used adjuvants, both in humans and animals, are mostly empirically developed, without a clear understanding of their cellular and molecular mechanisms of action. Recent research suggests that most, if not all, adjuvants enhance the response of T and B cells by involving components of the congenital immune

system rather than directly affecting the lymphocytes themselves (6, 7, 8, 9).
Researchers have found that a safe and powerful adjuvant to enhance humoral and cellular immune reactions induced by a protein-subunit can be a physical radio frequency. Physical adjuvants use physical energy rather than chemicals to stimulate tissue reactions and release endogenous responses to irritation to accelerate immunization. It has been shown that non-invasive radio-frequency can increase humoral and cellular immune responses induced by protein antigen with adjuvant effects comparable to those of commonly used chemical adjuvants (10).

THE PURPOSE OF THE STUDY
To check the stimulating properties of formalin in the immunization of animals in obtaining specific serum precipitating high titers to determine the type of blood.

MATERIAL AND RESEARCH METHODS
39 rabbits of both sexes of the Shinshilla breed weighing 3-3.5 kg were used in the experiments.

The animals were kept in standard conditions in accordance with the requirements of the methodical manual "Rules and methods of work with laboratory animals in experimental microbiological and immunological studies". Combined feed and juicy animal feed were given into cells. Animals were given purified water. Animals were kept under controlled environmental conditions (20-22°C and relative humidity of 50-70%). In the animal houses the lighting cycle was maintained for 12-14 hours. Air temperature and humidity were recorded daily. The animals were examined daily in the cage. The general condition of animals was fixed: peculiarity of behavior, intensity, character of motor activity; coordination of movements, tone of skeletal muscles; frequency and depth of respiratory movements; condition of hair and skin; quantity and consistency of faecal masses. As an antigen for the immunization of rabbits used a mixture of human blood serum and 1% formalin solution (group 1), a mixture of human blood serum and 5% formalin solution (group 2), a mixture of human blood serum and 10% formalin solution (group 3), in a ratio of 1:1, which was kept in the refrigerator at a temperature of 4-6 ° C for 72 hours. As a control, immunization of rabbits (5 in total) was performed with human blood serum and 0.9% saline solution (group 4).

Immunization of rabbits was carried out according to the scheme proposed by D.D.Jalalov and R.A.Hasanov (1997). The antigen is injected into the marginal vein of the rabbit's ear three times, in 1 day intervals in the volume of 1 ml/kg of the rabbit's weight. Blood samples of immunized animals are taken on the 4th, 7th and 9th day after the last injection. In the presence of pretsipitinov in the serum of rabbitstitre 1:5000 and 1:10000, the blood is taken with a heart puncture and bloodletting. After immunization, if the titre of serum precipitating serum did not reach the working titre, it was reimmunized two weeks after the last immunization, by a single administration of the antigen.

Blood sampling and serum production. The blood was taken from the marginal vein of the rabbit's ear (the ear was treated with alcohol before the blood was taken). Blood was placed in a thermostat at 37°C for 1 hour. (to separate the serum). Afterwards, the serum was sampled by centrifugation at 3000 rpm for 10 minutes. If necessary, the centrifugation time was increased. The serums were kept at 20°C in a mixture of 3% boric acid in a ratio of 3 mg/1 ml within a day.

RESEARCH RESULTS AND DISCUSSION.

Introduction of a mixture of human blood serum and 10% formalin solution resulted in immune response only in 60% of immunized animals after primary immunization, while introduction of a mixture of blood serum and 5% and 1% formalin solution, respectively, the percentage of animals with immune response reached 40% and 20%. After the reimmunization of animals with a mixture of serum and 10% formalin solution, a positive result was achieved in 90% of cases. When a mixture of serum with 5% and 1% formalin was administered, the percentage of positive results was 60% and 40%, respectively. At the same time, the duration of immunization was 22 days, the titre of immune serum precipitating obtained reached 1:5000 and 1:10000, no fatal cases in rabbits were observed.

The use of 10% formalin solution in immunization contributed to the production of rabbit immune sera precipitating human proteins with high specific activity in immunological reactions. Thus immunostimulating effect at absence of toxic influence on an animal, without occurrence of adjuvant illness, reached 90% of rabbits.

After the first introduction of antigens appearance of animals from experimental groups did not differ from the appearance of animals from the control group. After the second administration a slight decrease in appetite and mobility of animals was noticed. After the third administration of the antigen, some animals were observed to have hyperemia at the injection site and heart rate increased.

After reimmunization the behavior of experienced animals was kept as sluggish in comparison with control animals, but no serious changes in behavior and appearance were observed.

Blood is taken and the antibody titer and specificity of the serum obtained are determined. Precipitating serum is considered suitable for

forensic medical research, if it has a titre 1:5000 and 1:10000, ie when adding it to the homologous normal serum, diluted in 10,000 times, the sediment falls within 10 minutes and it does not give the sediment of normal serum of other species, diluted in 1000 times within one hour.

Table 1: Titre and time of immune serum precipitation reaction

Groups	No of rabbits	Titre/precitation time			
		Primary immunization		Reimmunization	
1st:	1	1:5000	+40 sec.	-	-
	2	1:1000	+1 min.	1:5000	+ 1 min.
	3	1:1000	+3 min.	1:5000	+ 3 min.
	4	1:5000	+6-8 min.	-	-
	5	1:100	+20 sec.	1:1000	+ 6 min.
	6	1:5000	+4 min.	-	-
	7	1:10000	+3 min.	-	-
	8	1:5000	+5 min.	1:5000	+ 5 min.
	9	1:10000	+2 min.	-	-
	10	1:1000	+6 min.	1:5000	+ 4 min.
2nd:	1	1:5000	+2-3 min.	-	-
	2	1:1000	+6 min.	1:1000	+ 2 min.
	3	1:100	+4 min.	1:1000	+ 6 min.
	4	1:100	+4 min.	1:5000	+ 4-6 min.
	5	1:100	+1 min.	1:1000	+ 6 min.
	6	1:10000	+6 min.	-	-
	7	1:5000	+3 min.	-	-
	8	1:5000	+5 min.	-	-
	9	1:100	+40 sec.	1:1000	+ 1 min
	10	1:1000	+2 min.	1:5000	+ 5 min.
3rd:	1	1:100	+1-2 min.	1:100	+ 3 min.
	2	1:1000	+6 min.	1:5000	-
	3	1:100	+4 min.	1:1000	+ 2 min.
	4	1:1000	+3 min.	1:5000	+ 5 min.
	5	1:100	+1-2 min.	1:100	+ 1 min.
	6	1:5000	+6 min.	-	-
	7	1:100	+1 min.	1:1000	+ 3 min.
	8	1:5000	+5 min.	-	-

	9	1:100	+1 min.	1:100	+ 2 min.
	10	1:5000	+6 min.	-	+ 4 min.
4th: (control)	1	1:1000	+6-8 min.	1:5000	+ 7 min.
	2	1:100	5-6 min.	1:100	+ 6 min.
	3	1:100	+3 min.	1:1000	+ 3-4 min.
	4	1:100	+4-6 min.	1:100	+ 4 min.
	5	1:100	+ 6 min.	1:1000	+ 8 min.

The table shows that the titre of antibodies in the first group corresponded to 1:10000 in 2 cases and 1:5000 in 4 cases. In the second group, there were 3 cases of 1:5000 and 1:10000 in the second group; in the third group, there was not 1:10000 in the third group, but 1:5000 in the third group; in the fourth (control) group, there was 1:1000 in the fourth group and 1:100 in the fourth group, which was not suitable for forensic use.

All working titre serum precursors obtained were found to be specific, i.e., 1:1000 dilution within 1 hour of the precipitation reaction, except for the species concerned, did not occur with other antigens.

Thanks to the use of the immunostimulating effect of 10% formalin, the duration of the immunization process (22 days) and the production of a highly specific serum were significantly reduced, while the yield of the target product was increased by increasing antibody production in animals with simultaneous reduction of labour costs. It should also be noted that after immunization one-time reimmunization, the percentage of positive results was increased in 90% of cases.

Conclusions

Thus, new effective approaches to obtaining heteroimmune serum based on an optimal combination of protein antigens in combination with a 10% formalin solution, providing a high immune response in 90% of animals, a significant reduction in the timing of immunization, material and labor costs.

The obtained heteroimmune precipitating serums are highly specific and are considered suitable for use in forensic medicine.

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	5	1:100	+20 sec.	1:1000	+ 6 min.
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	3	1:100	+4 min.	1:1000	+ 2 min.
	4	1:1000	+3 min.	1:5000	+ 5 min.
	5	1:100	+1-2 min.	1:100	+ 1 min.
	6	1:5000	+6 min.	-	-
	7	1:100	+1 min.	1:1000	+ 3 min.
	8	1:5000	+5 min.	-	-

	9	1:100	+1 min.	1:100	+ 2 min.
	10	1:5000	+6 min.	-	+ 4 min.
4th: (control)	1	1:1000	+6-8 min.	1:5000	+ 7 min.
	2	1:100	5-6 min.	1:100	+ 6 min.
	3	1:100	+3 min.	1:1000	+ 3-4 min.
	4	1:100	+4-6 min.	1:100	+ 4 min.
	5	1:100	+ 6 min.	1:1000	+ 8 min.

The table shows that the titre of antibodies in the first group corresponded to 1:10000 in 2 cases and 1:5000 in 4 cases. In the second group, there were 3 cases of 1:5000 and 1:10000 in the second group; in the third group, there was not 1:10000 in the third group, but 1:5000 in the third group; in the fourth (control) group, there was 1:1000 in the fourth group and 1:100 in the fourth group, which was not suitable for forensic use.

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