

## AGE-INDUCED DIFFERENCES IN ANTIGENIC PROPERTIES OF SKIN

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One of the main factors preventing the survival of transplants in homoplastic skin grafts is their biological incompatibility with the organism of the recipient due to the difference in antigenic properties.

Several investigators have indicated in their papers that the skin of newborn animals and embryos possesses the weakest antigenic properties [2,4-9]. This has strengthened the view that skin from embryos, newborn, or infant corpses should be used for homoplastic grafts [1,3].

In 1957, in order to investigate the changes in the antigenic properties of skin with age, we carried out experiments on rabbits by means of the complement fixation reaction (CFR).

### Experimental Method

The experiments were performed on 15 adult rabbits, which were divided into three groups according to sex and weight.

Monospecific sera against the skin of a 28-day embryo, a 7-day-old, and an adult rabbit were obtained in the following way: The five rabbits of the first group were given injections of extract (antigen) of skin from a 28-day-old embryo; the five rabbits of the second group were given injections of extract (antigen) of skin from a 7-day-old rabbit; the five rabbits of the third group received injections of extract (antigen) of skin from an adult rabbit. Six other adult rabbits received no antigens, and these rabbits served as a control for determination of the specific antibodies to the antigens under investigation. These animals were kept in the same conditions along with the experimental rabbits.

The material (skin) for immunization was taken from a 28-day-old embryo, a 7-day-old rabbit, and an adult rabbit. The skin was taken from the back of the rabbits after removal of the hair. The obtained material was shredded with scissors and ground up with powdered glass in a hypertonic solution of common salt (10 ml of salt solution in concentration 1.7% per 1 g of tissue) in a marble mortar. The suspension stood for two hours, after which it was centrifuged for 15 min at 2500 rpm. The centrifuged liquid (antigen) was diluted with distilled water to the concentration of physiological saline. This extract (antigen) was poured in 10-15 ml batches into penicillin ampoules, preserved at 0°, and used for immunization of the experimental animals and the CFR tests.

The whole immunization process took four weeks. In the first three days, the rabbits were given an intravenous injection of the antigen (extract) of the skin of the 28-day-old embryo, the 7-day-old, or adult rabbit in a dose of 1 ml at dilution 1:60. After a four-day interval, a second course of immunization was carried out, 1 ml being injected intraperitoneally on the first day and 1 ml intravenously on each of the following two days. Three courses of this second type were given. On the eighth day after the last intravenous injection of antigen, a CFR test with the corresponding antigen was carried out on the blood serum of the immunized and control animals.

# Results of CFR Test of Age-Dependent Antigenic Properties of Rabbit Skin

Expt. No.	No. of animal used for serum test	Antigen	Control (undiluted sera without antigen)	Serum dilutions					
				1:10	1:20	1:40	1:80	1:160	1:320
1	1	Skin extract from 28-day-old embryo	H	+	+	+	H	H	H
	2		H	++	++	+	+	H	H
	3		H	++	++	+	H	H	H
	4		H	+	+	+	H	H	H
Control rabbits	14-15		H	H	H	H	H	H	H
2	5	Skin extract from 7-day-old rabbit	H	+++	+++	++	++	++	+
	6		H	++	++	+	+	H	H
	7		H	++	++	+	+	+	H
	8		H	+++	+++	+++	+++	++	++
Control rabbits	9		H	++	++	++	++	+	+
	16-17		H	H	H	H	H	H	H
3	10	Skin extract from adult rabbit	H	+	H	H	H	H	H
	11		H	+	H	H	H	H	H
	12		H	+	H	H	H	H	H
	13		H	+	+	+	H	H	H
Control rabbits	18-19		H	H	H	H	H	H	H

Notes: 1. Results of complement fixation reaction indicated by conventional method: ++++ no hemolysis; H—complete hemolysis; +++, ++, + intermediate stages of hemolysis. 2. Antigens for CFR test were taken in dilution 1:50. 3. The CFR was determined 45 min after start of test.

To determine the immunizing dose of antigen, we took nine rabbits (not previously immunized), and gave them an intravenous injection of the specific antigen at various dilutions (1:20, 1:40, 1:60).

When the rabbits received an intravenous injection of antigen obtained from the skin of the embryo and 7-day-old rabbit in dilutions 1:20 and 1:40, the animals died. When the animals received an intravenous injection of extract prepared from adult rabbit skin in the same dose at dilution 1:20, we observed a reaction in the form of temporary convulsions; an injection of antigen in dilution 1:40 produced no reaction in the animals.

In view of the high toxicity of skin antigens from the embryo and 7-day-old rabbit, all three groups of experimental animals were accordingly immunized with antigens in dilution 1:60. The CFR test was conducted by the conventional method on the eighth day after the fourth course of immunization. By means of this test we determined the antigenic properties of the skin of a 28-day embryo, a 7-day-old, and an adult rabbit from the antibody content of the sera of the experimental animals.

## Experimental Results

As the given table shows, the highest CFR titer was found in experimental animals immunized by the skin extract from the 7-day-old rabbit. In this group (second series) we observed a positive CFR (from + to +++) in all five rabbits with serum dilution 1:80, and a weakly positive reaction (from + to ++) in three rabbits with serum dilution 1:320.

In the first series (four rabbits), where the animals were immunized by an extract of embryonic skin, the CFR for all the rabbits was weakly positive (+) at serum dilution 1:40 and, in one rabbit, a weakly positive reaction (+) was noted at serum dilution 1:80.

In the third series (four rabbits), where the animals were immunized with extract from the skin of the adult rabbit, the antibody titer was low. In all four rabbits, the CFR was positive (+) only at serum dilution 1:10, and in one rabbit at dilution 1:40, while in the other three rabbits the CFR at this dilution was negative.

The reliability of the CFR in all the experiments was confirmed by the negative reaction obtained in control tubes, where the serum was undiluted and the corresponding antigens were not added. In addition, to check

the reliability of the results we conducted CFR tests with the serum of animals of the control group. This test was also negative.

The results of the CFR tests showed that the highest titer was obtained in the group of rabbits which were immunized with the skin extract from the 7-day-old rabbit. The antibody titer was half as high in rabbits immunized with the extract from embryonic skin.

The lowest CFR titer was obtained in the group of experimental animals immunized with skin extract from the adult rabbit.

In view of the results of earlier experiments which showed a reduction of antigenic properties of skin at low temperatures [10], we decided to test the effect of low temperatures (0°) on antigen extracts preserved for four weeks. For this purpose we took six nonimmunized rabbits and repeated the experiment to establish the immunizing dose of antigen (as before, 1 ml in dilutions 1:20 and 1:40 was injected intravenously).

When the rabbits received an injection of embryonic skin extract in dilution 1:20, there were no deaths. Only slight, quickly passing convulsions were noted. An injection of skin extract from the 7-day-old rabbit in dilution 1:20 caused the death of the animals; on injection of the extract in dilution 1:40 no shock effects were observed. When skin extract from an adult rabbit in dilution 1:40 was injected, there were no shock effects in the rabbits.

An analysis of the results obtained in the experiments to determine the immunizing dose at the beginning and end of our investigation, as also the results of the CFR test (low positive indices of this reaction in all three series), indicates the lowering of the antigenic properties of all the extracts due to four week's exposure to low temperature (0°), with the greatest loss of antigenic properties being found in the extract of embryonic skin.

#### SUMMARY

The antigenic differences of the skin of a 28-day-old rabbit embryo, a 7-day-old, and adult rabbit was studied by means of the complement fixation reaction. The specific antigenic properties of the skin were found to be most pronounced in a 7-day-old rabbit, a little less so in the embryo, and the least in the adult rabbit.

Storage of skin extracts for a period of one month at zero temperature reduced their antigenic properties. This reduction was greatest in the embryonic skin extract (antigen).

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