

# IMMUNOPHYSIOLOGICAL CHANGES IN HOMOTRANSPLANTATION OF DOG EXTREMITIES

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A true take of a homotransplanted extremity has been obtained only for rats [6, 7, 12] with the use of parabiosis. For dogs such operations have resulted in death of the recipient or rejection of the transplant. The maximal life span of homoplastically transplanted dog extremities was 17-40 days [1, 2, 4, 10-12].

It was established that 1-3 days after transplantation, the muscle of both auto- and homotransplanted extremities experienced excitation under the effect of an electrical current. Later, after several days of the absence of excitability, the latter is restored in the autoplastically transplanted extremity but does not appear in the homotransplanted extremity before its rejection. The absence of the excitability of muscles of the homoplastically transplanted extremity is considered to be the consequence of the biological incompatibility of the tissues of the donor and recipient [3, 4].

The problem of the demonstrations of transplantation immunity in homotransplantation of an extremity has not been studied. The attempts of certain investigators [5] to detect serum hemagglutinins in the blood of recipient dogs proved unsuccessful.

The task of the present investigation was to compare the physiological picture of the state of a homotransplanted extremity of dogs with the indexes of transplantation immunity (data of the hemagglutination reaction).

## EXPERIMENTAL METHOD

Homotransplantation of the extremity was carried out simultaneously on 2 dogs under morphine-amobarbital sodium anesthesia. The extremity of the donor was separated at the level of the middle 3rd of the femur. The main femoral vessels were connected by a mechanical vascular suture. The time of excluding the extremity from circulation was 30-79 min.

After the operation we studied the general condition of the dogs: we counted the number of heart-beats and respiration per min, measured the rectal temperature, and measured the skin temperature of the hind legs. By means of a ISE-01 electron stimulator we investigated the excitability of the muscles (rheobase, chronaxie, the constant of short-term excitability, and the strength-duration curve).

All investigations were carried out before the operation and daily after the operation up to the moment of death of the animal or transplant.

By means of the hemagglutination reaction in a saline medium we established the compatibility of the donor and recipient blood. Every 3 days after the operation we determined the complete and incomplete hemagglutinins (the latter by means of treating the erythrocytes with 0.1% solution of trypsin at pH 7.2). To carry out the reaction 1 drop of 2% suspension of donor erythrocytes was added to 2 drops of recipient serum in test tubes in a dilution of 1:2-1:32. The reaction was set up at 18-19 and 37°. Thirteen min after the start of setting up the reaction the contents of the test tubes were centrifuged for 1 min. The character of erythrocyte sedimentation made it possible to judge the degree of their agglutination.

Titer of Serum Hemagglutinins of Recipient Dogs at Various Periods After Homotransplantation of Extremity

Name of dog	Type of antibodies	Temp. (in degrees)	Titer of antibodies				Period of death of recipient or transplant	
			Be-fore operation	After operation				
				3 days	6 days	9 days	12 days	
Seryi	Complete	37	—	—	—	1:2		14th day. Death of transplant
	Incomplete	18—19	1:2±	—	1:4	1:16		
		37	—	1:2	1:2			
		18—19		1:4±	1:2	1:16		
Barbos	Complete	37	—	—	—	—	1:8	13th day. Death of recipient (from hemorrhage)
	Incomplete	18—19	—	—	—	—	1:8	
		37	—	—	—	—	1:8	
		18—19	—	—	1:2	—	1:8	
Pestryi	Complete	37	1:8	1:4±	1:16	1:2±		10th day. Death of recipient
	Incomplete	18—19	1:32	1:8	1:16	1:8		
		37	—	1:16	1:16	1:2		
		18—19	—	1:32	1:32	1:8		
Pushok	Complete	37	—	—	—	—		9th day. Death of transplant
	Incomplete	18—19	—	—	—	—		
		37	—	1:2	—	1:2		
		18—19	—	1:2	—	1:2		
Mal'va	Complete	37	—	—				6th day. Death of transplant
	Incomplete	18—19	—	—				
		37	—	1:4				
		18—19	—	1:4				
Bobik	Complete	37	—	1:4				6th day. Death of transplant
	Incomplete	18—19	—	1:8				
		37	—	—	1:4			
		18—19	—	1:4	1:2			
Strela	Complete	37	—	—				6th day. Death of recipient.
	Incomplete	18—19	1:2	—				
		37	—	—				
		18—19	—	1:4				

Designations: absence of agglutination of erythrocytes; ± doubtful result.

The experiments were carried out on 8 mature dogs.

#### EXPERIMENTAL RESULTS

A study of the characteristics of the general state of the animal, its life span, the character of primary take of the transplant made it possible to establish a certain dependence of these indexes on the degree of blood compatibility of the donor and recipient. The blood of 3 recipients out of 8 was incompatible to the donor blood. In one of them immediately after the operation we noted a drop of the skin temperature of the transplant to the level of the room temperature and the absence of pulsation of the vessels in it. The cause for possible thrombosis of the leg vessels could have been trauma of the intima of the artery which occurred during collapse of the artery during the operation. Two other animals with blood incompatibility died on the 5-10th day in spite of the viability of the transplant. For 2-3 days before death of the animals we observed a deterioration of their general state (areactivity, muscular debility, an increase in heart-beat to 160-170 per min, vomiting, the appearance of a loose tarry stool).

In four out of five cases of blood incompatibility we observed necrosis of the transplanted extremity on the 6-14th day. One animal died from hemorrhage from the vessels of the transplanted extremity.

From the 2-3rd day postoperation the dogs walked on 3 paws, their appetite was poor. During the first 3-4 days we noted an increase in the heart-beat by 30-40 per min, and a slowing down of respiration. At first the transplanted leg did not differ outwardly from an autotransplanted leg [4, 8, 11]. From 1 to 3 days after the operation the transplanted leg became edematous, its skin was hot to the touch, hyperemic, and the skin temperature was 5-13° higher than that of the healthy hind leg. In all cases we noted hair growth. On the 3-4th day we observed the escape of lymph from the integument, on the 6-9th day foci of purulent ulceration of the skin and its exfoliation in layers, and loss of hair. The temperature of this extremity dropped to the level of room temperature and after 1-2 days the extremity was rejected.

The excitability of the muscles of the transplanted extremity persisted for 1-3 days postoperation in 7 dogs. The rheobase was measured as 22-34 V (before transplantation, 3-10 V). It was not possible to establish any regularity in the changes of chronaxie. The strength-duration curve was determined at this period; on subsequent days this could not be done owing to an increase of chronaxie. For the next several days irritability was absent, and on the 5-8th day it appeared again. The rheobase during these days was less than on the first day; chronaxie was either not determined at all or was very high, up to 25 m/sec. The constant of short-term excitability greatly exceeded the preoperation level. This restoration of muscle irritability indicates the possibility of not only a central but a local regulation of the vitality of the tissues of the homotransplanted extremity.

The hemagglutination reaction demonstrated the appearance of complete and incomplete hemagglutinins in the recipient serum in a titer of 1:2-1:32 beginning on the 3rd day after homotransplantation (see table). As a rule the incomplete antibodies were elicited in the larger titer. A temperature of 18-19° in our experiments was more suitable for eliciting immune hemagglutinins. The most intense accumulation of antibodies was noted in the serum of recipients whose blood was incompatible with the blood of the donors. The rise in the antibody titer was observed by the 6-9th day after transplantation, which coincided with the onset of the homotransplant necrosis.

In one case (the dog Pestryi) we observed a certain drop of the titer of hemagglutinins after transplantation as compared with the preoperation level. This phenomenon is possibly associated with the marked exhaustion of the protective forces of the organism in connection with the entrance into the blood of the recipient of an enormous quantity of antigenic material. Therefore, in this case, death of the recipient with viability of the transplant was observed.

## SUMMARY

Homotransplantation of the left posterior extremity was carried out in 8 adult dogs. In the presence of the blood incompatibility the recipient died on the 5th-10th day after the operation. In the rest of the cases the transplant became detached on the 6th-14th day. The excitability of the muscles of the transplanted extremity may be restored after the degeneration of the neuromuscular synapses. The capacity of the homotransplanted extremity for direct muscular excitability is an important compensatory reaction of the transplanted organ. Primary "take" of the extremity was accompanied by the accumulation of complete and incomplete hemagglutinins in the recipient's serum from the 3rd day after the transplantation (1:2-1:32 titers). A rise in the antibody titer was observed by the 6th-9th post-transplantation day which coincided in time with the onset of the homotransplant necrosis.

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All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.

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