

DETECTION OF INCOMPLETE ANTIBODIES IN DOGS AFTER  
AFTER HOMOPLASTIC TRANSPLANTATION  
OF VARIOUS ORGANS

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Autoplastic transplantation of tissues such as the skin is usually a successful procedure and is widely used in plastic operations, whereas homografts as a rule die after a varying period of time after transplantation.

The reason for the different behavior of auto- and homografts is thought by many workers to be immunological differences between the tissues of the donor and recipient [1, 4, 5, 9, 12, 16 and others].

It has been shown that human erythrocytes contain about 40 different antigens, by means of which 9 immunological systems can be differentiated in man, comprising more than 300,000 individual antigenic variations. A diversity of antigenic properties has also been demonstrated for human tissues [5].

The antigenic composition of the cells and tissues of animals has received less study. There are, however, many experimental findings which give evidence of the complexity of the antigenic structure of animal tissues and of the wide variety of the antigens in animals of the same species. For instance, during the study of the antigenic structure of the dog's red cells it was found that at least four different agglutinogens were present therein [6, 11, 13, 15, 17, 18].

The existence of antigenic differences between the tissues of dogs was confirmed by experiments on repeated homotransplantation of kidneys. It was found that secondary kidney homografts died more quickly than primary, as a result of immunization of the recipient during the primary grafting [10, 16, and others].

Attempts have been made to detect specific antibodies to the donor's antigens, arising in the recipient as a result of homotransplantation. Serological investigations of dogs' sera have given contradictory results.

Yu. Yu. Voronoi [2, 3], for instance, reported the appearance of complement-fixing antibodies in the serum of dogs after grafting of a kidney.

Curtiss and Herndon [8] found agglutinins to the donor's erythrocytes in the serum of 2 of 5 dogs after homoplastic transplantation of a joint.

Simonsen [16], on the other hand, could find neither hemagglutinins nor complement-fixing antibodies in the serum of dogs after transplantation of kidneys.

Greater success was obtained in the investigations of Simonsen [16] and of Muirhead and Groves [14], when they used the agglutination reaction in a protein medium and the Coombs' test [7], for they were able to detect incomplete antibodies to the donor's erythrocytes in the serum of dogs after grafting operations.

The present experimental research was devoted to the immunological study of the sera of dogs after the homoplastic transplantation of different organs.

TABLE 1

Group of dogs	Character of homoplastic operation performed	No. of dogs examined	
		before operation	after operation
First	Grafting of a second heart with a lung or the lobe of a lung	11	5
Second	Grafting of a kidney with or without removal of the animal's own kidney	5	5
Third	Grafting of the lobe of a lung	5	2
Fourth	Creation of a crossed circulation between donor and recipient	4	4
Fifth	Complete replacement of heart and lungs, grafting of limbs or parts of the body of a puppy to an adult dog, grafting of the knee joint, and so on.	13	3
	Total number investigated	38	19

## METHOD

The object used for the immunological study was the sera and erythrocytes of recipients and donors both before and at various intervals after operation.\*

According to the character of the operations performed on them, the recipient dogs could be divided into five groups.

It will be clear from the details given in Table 1 that the blood of 38 pairs of donors and recipients was examined before operation. Because of the complicated nature of the operations, half the recipients died within 48 hours of operation, and so their blood was not reexamined. The serum and erythrocytes of 19 recipients whose period of survival after operation was longer than four days were tested in immunological reactions in order to detect the changes accompanying homografting.

In performing the investigations two methods were used:

1) the agglutination reaction in a saline medium [2 drops of serum (whole and diluted) + 1 drop of a 3-5% suspension of erythrocytes in physiological saline, with subsequent centrifugation for 1 minute at 2000 rpm];

2) the agglutination reaction in a protein medium [2 drops of whole or diluted serum were poured out, and then to each tube was added 1 drop of a 3-5% suspension of erythrocytes in a 20% solution of albumin. The tubes were shaken and allowed to stand for 1.5-2 hours at room temperature. To prepare the 20% albumin solution, 1 g of dry albumin from human serum was dissolved in 5 ml of 0.85% NaCl solution, after which the mixture was rendered alkaline (to pH 7.2) by the addition of a few drops of 10% NaOH solution].

The results of the hemagglutination reaction in a protein medium were read by means of a hand lens over a pearl electric light.

## RESULTS

In Table 2 we give the results of the immunological investigation of the sera of 17\*\* recipients with the donors' erythrocytes at various intervals after homografting operations.

It is clear from Table 2 that during the investigation of the recipients' sera by the agglutination reaction with the donors' erythrocytes in a saline medium, only in the dog Urs was it possible to detect agglutinins to the donor's erythrocytes on the tenth day after operation.

In the other cases no agglutinins to the donor's erythrocytes were found by this method in the recipients' serum, both before and at different intervals after the homoplastic operations.

We obtained more successful results by using the agglutination reaction with the donor's erythrocytes in a

\*The operations were performed by V. P. Demikhov in the laboratory for transplantation of organs.

\*\*The results of investigation of the sera of two recipients, dying on the fifth day after transplantation of the head of the femur, are not given in the table. No changes were found in the sera of these dogs.

TABLE 2

Results of the Reaction of Agglutination of the Donors' Erythrocytes by the Recipients' Sera after Homoplastic Transplantation Operations

Recipient	Character of homo- transplantation operation	Length of survival after operation (in days)	Time of investigation of serum	Method of investigation																			
				agglutination reaction in saline solution		agglutination reaction in protein solution																	
				Dilutions of sera																			
				whole	1:2	1:4	1:8	1:16	1:32	whole	1:2	1:4	1:8	1:16	1:32								
Starik	Grafting of a second heart and a lung	10	Before operation On 7th day after operat. On 9th day after operat.	-	-	-	-	-	-	-	-	+	++	++	++	++	++	++	++	++	++	++	++
Stroinyi	Grafting of a second heart and of a lobe of a lung	14	Before operation On 7th day after operat. On 10th day after operat.	-	-	-	-	-	-	-	-	+	++	++	++	++	++	++	++	++	++	++	++
Urs	Grafting of second heart and a lung	13	Before operation On 10th day after operat.	++	++	-	+	-	-	-	-	++	++	++	++	++	++	++	++	++	++	++	++
Zheltyi	The same	8	Before operation On 4th day after operat. On 6th day after operat.	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+
Khromoi	»	5	Before operation On 5th day after operat.	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+
Buket	Grafting of a kidney after removal of the recipient's own kidneys	11	Before operation On 5th day after operat. On 11th day after operat.	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+
Chernysh	Grafting of a kidney	45	Before operation On 8th day after operat. On 19th day after operat. On 32nd day after operat. On 45th day after operat.	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+

TABLE 2 (continued)

Recipient	Character of homotransplantation operation	Length of survival after operation (days)	Time of investigation of serum	Method of investigation											
				agglutination reaction in saline solution				agglutination reaction in protein solution							
				Dilutions of sera											
				whole	1:2	1:4	1:8	1:16	1:32	whole	1:2	1:4	1:8	1:16	1:32
Polkan	Grafting of a kidney	9	On 6th day after operat.	—	—	—	—	—	—	++	++	—	—	—	—
Chernyi	The same	14	Before operation On 8th day after operat. On 12th day after operat.	—	—	—	—	—	—	±	±	—	—	—	—
Tuzik	The same	9	Before operation On 5th day after operat.	—	—	—	—	—	—	±	±	—	—	—	—
Lokhmach	Grafting of the lower lobe of a lung	45	Before operation On 4th day after operat. On 11th day after operat.	—	—	—	—	—	—	+	+	—	—	—	—
Recipient No. 2	The same	11	Before operation On 6th day after operat.	—	—	—	—	—	—	±	—	—	—	—	—
Kerzon	Grafting of a knee joint	more 12	Before operation On 11th day after operat.	+	±	—	—	—	—	+	+	±	—	—	—
Dog No. 1	Creation of a crossed circulation	9	Before operation On 5th day after operat. On 7th day after operat.	—	—	—	—	—	—	+	+	±	—	—	—
Dog No. 2		9	Before operation On 5th day after operat. On 7th day after operat.	—	—	—	—	—	—	—	+	+	—	—	—

TABLE 2 (continued)

Recipient	Character of homo- transplantation operation	Length of sur- vival after operation (days)	Time of investi- gation of serum	Method of investigation											
				agglutination reaction in saline solution				agglutination reaction in protein solution							
				Dilutions of sera											
				whole	1:2	1:4	1:8	1:16	1:32	whole	1:2	1:4	1:8	1:16	1:32
Ryzhii	Creation of a crossed circulation	4	Before operation On 4th day after operat.	—	—	—	—	—	—	—	—	—	—	—	—
Malyshev		4	Before operation On 4th day after operat.	—	—	—	—	—	—	—	—	—	—	—	—

Conventional signs: +++ precipitate of erythrocytes fills the whole of the bottom of the tube in the form of an umbrella with winding edges, sometimes marked with radial stripes; ++ the precipitate fills almost the entire bottom of the tube and clumps of agglutinated erythrocytes are seen; + part of the erythrocytes appear as a homogeneous precipitate, the rest in the form of clumps or agglutinates. The edge of the precipitate is uneven. The surface is rough. (+) The greater part of the erythrocytes appear as a homogeneous precipitate and there are a few clumps. ± Doubtful agglutination. The edges of the precipitate are not quite smooth. — Erythrocytes are evenly deposited at the bottom of the tube; a homogeneous precipitate with a clear, smooth border.

20% albumin solution. By using this method we were able to demonstrate the presence of agglutinins to the donor's erythrocytes in several recipients and to observe an increase in their titer after homografting operations.

A second heart was grafted on to the dog Starik, with a lung in the thorax. The dog survived for ten days after operation.

It can be seen from Table 2 that before operation agglutinins to the donor's erythrocytes were found in the recipient's serum to dilutions of 1 : 4. On the seventh day after operation the titer of agglutinins to the donor's erythrocytes was increased. There was a well-marked reaction of agglutination of the donor's erythrocytes to a dilution of the serum of 1 : 32. The same results were obtained on the ninth day. The recipient's serum did not agglutinate its own erythrocytes.

The serum of the dog Stroinyi before operation agglutinated the donor's erythrocytes in albumin solution only in the undiluted form. On the seventh day after grafting of the second heart with a lobe of the lung into the thorax, the agglutinin titer rose to a dilution of 1 : 4, and on the tenth day after operation a clear reaction of agglutination of the donor's erythrocytes took place to a dilution of 1 : 16.

In the serum of the dog Urs, into whose thorax a second heart, with a lung, was grafted, in a saline medium agglutinins to the donor's erythrocytes were found in a titer of 1 : 8 on the tenth day after operation.

When investigated in a protein medium, the agglutination reaction was more clearly apparent and the agglutinin titer was higher (to a dilution of 1 : 16). No differences were found, however, in the agglutinin titer before and on the tenth day after operation.

A donor's kidney was grafted on to the dog Buket after its own kidneys had been removed. The recipient died from uremia on the 11th day after operation. It is clear from Table 2 that the recipient's serum before operation and on the fifth day after operation feebly agglutinated the donor's erythrocytes in the undiluted form and in a dilution of 1 : 2. On the 11th day after operation agglutinins to the donor's erythrocytes were found in the recipient's serum to a dilution of 1 : 16, whereas the recipient's own erythrocytes and the erythrocytes of other dogs were agglutinated by the serum only to a dilution of 1 : 4.

Grafting of a decapsulated kidney was performed on the dog Chernysh, without removal of the animal's own kidneys. The dog remained in a satisfactory condition. On the 45th day after operation an exploratory laparotomy was performed. The grafted kidney was found to be enlarged four- or fivefold and the renal artery was thrombosed. It is evident from Table 2 that the recipient's serum before operation and on the eighth day after operation did not agglutinate the donor's erythrocytes. Only on the 19th day after operation were agglutinins to the donor's erythrocytes found in whole serum, their titer rising very slowly to 1 : 4 on the 45th day.

Agglutinins to the donor's erythrocytes were found to a dilution of 1 : 2 in the serum of the dog Polkan on the sixth day after homoplastic transplantation of a kidney. Unfortunately this serum was not tested before operation.

It is clear from Table 2 that the appearance of agglutinins was not observed in all the animals.

The titer of agglutinins to the donor's erythrocytes was not increased in the recipients Zheitzki and Khromoi, whose sera were tested on the 4th-6th day after operation.

In the serum of the recipient Chernyi, tested on the eighth and 12th day after operation, and that of the recipient Tuzik, tested on the fifth day after operation, agglutinins to the donor's erythrocytes were absent.

The dog Lokhmach, on which the operation of grafting of a lower lobe of the donor's lung was performed, survived for 45 days after operation. When it was examined post mortem, the grafted lobe was found to be almost completely absorbed. Since the donor was not kept alive, no immunological investigations were carried out after the 11th day. Tests of the serum on the fourth and 11th day after operation showed the absence of agglutinins to the donor's erythrocytes.

No increase in the agglutinin titer was observed in the dog Kerzon on the 11th day after grafting of a knee joint, or in recipient No. 2 after grafting of a lung. No significant changes were found, moreover, in the sera of two pairs of dogs after the creation of a crossed circulation.

Immune agglutinins were thus found in the serum of five recipient dogs on which a heart and lung, or a kidney had been grafted, out of the 19 dogs investigated after various homografting operations, in a titer of between 1 : 4 and 1 : 32, to the erythrocytes of the corresponding donors, the appearance of which may be accounted for by immunization of the recipient with antigens of the donor's tissues.

These agglutinins evidently belong to the group of incomplete antibodies, for they are best detected in a protein medium by the conglutination method.

The absence of immunological changes in the serum of the majority of recipients after homografting operations may evidently be explained by the fact that the sera were tested on the fourth, the fifth and, only in a few cases, on the sixth day after operation, whereas the immunological reaction of the body to the introduction of foreign antigens is known to develop only seven or more days after the introduction of these antigens.

In some cases, despite the comparatively long survival period of the recipient after grafting of the organs, no antibodies to the donor's erythrocytes could be found in its serum. These cases may be explained by the peculiarities of the immune reaction of the recipient, by the specific features of the antigenic structure of the grafted organs, and also, possibly, by the close resemblance between the antigenic properties of the tissues of donor and recipient.

#### SUMMARY

Comparative immunological examination of sera obtained from recipient dogs before transplantation and at various periods after the homotransplantation of various organs demonstrated that immune agglutinins to the donor's erythrocytes may appear in the recipients' sera. These agglutinins belong to the group of incomplete antibodies, since they are best detected by the conglutination method. The appearance of hemagglutinins in the serum of recipients points to antigenic differences between the donor's and recipient's tissues has the basis of tissue incompatibility.

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