

# EXPERIMENTAL BIOLOGY

## A COMPARATIVE STUDY OF HUMORAL AND CERTAIN MORPHOLOGICAL CHANGES RESULTING FROM HOMOTRANSPLANTATION OF SKIN

E. A. Zotikov

From the Antigen Biology Laboratory (Director: Prof. P. N. Kosyakov) of the Institute of Experimental Biology  
(Director: Prof. I. N. Maisky) of the Acad. Med. Sci. USSR, Moscow

(Received for publication February 5, 1957. Presented by Active Member of the Acad. Med. Sci. USSR  
N. N. Zhukov-Verezhnikov)

At the present time little doubt remains about the problem of the immunobiological basis of death of the homograft. There is a great deal of evidence that cellular factors of the recipient play a leading part in tissue incompatibility (N. N. Elansky [3]; I. Khutnaya [5]; Darcy [8]; Simonsen, Buemann, Hammeltoft and others [13].

There is also more evidence in the literature that antibodies appear in the serum of the recipient after homotransplantation (E. A. Zotikov [4]; D. Amos and others [6]; P. Gorer [9]; E. Muirhead and M. Groves [11]).

However the problem of the relation of these antibodies to the survival or death of the homograft has not yet been thoroughly studied.

In this work we set out to investigate whether these antibodies take part in the creation of tissue incompatibility. For this purpose, along with a study of the serological properties of the recipients' sera, we made a capillaroscopic investigation in vivo of the transplanted skin grafts.

There are descriptions in the literature of the vascular changes occurring in transplanted skin grafts (I. Lehrfeld and A. Taylor [10]; R. Scothorne and I. McGregor [12]; L. Converse and F. Rapoport [7] and others).

These authors point out that at first in the transplanted skin graft there occurs restoration of the circulation by anastomosis formation between the vessels of the recipient and those of the graft. Later on stasis and thrombosis develop in the homografts, accompanied by destruction of the grafts themselves. This work is purely morphological in character and is not concerned with changes brought about by the humoral factors of the recipient.

For our research we made use of the ordinary capillaroscope or the MBS-2 binocular loupe system. Capillaroscopic examinations were undertaken from the 2nd-4th day after grafting, at intervals of 1-2 days, until either final cessation or complete survival of the circulation of the graft. On capillaroscopic examination of 4 cases, owing to impossibility of adequately illuminating the skin to see the superficial vascular network of the graft, we scarified the epidermis slightly with a razor. If circulation was present in the graft it rapidly filled with blood.

For serological examination we used the ordinary hemagglutination reaction in tubes.

### EXPERIMENTAL METHOD AND RESULTS

Experiments were performed on 35 rabbits weighing 1750-3500 g, mainly of the chinchilla variety, which were grafted with full thickness skin flaps measuring 50-100 cm<sup>2</sup>. Homografting was used with 22 animals, autografting in 13 animals and in 6 rabbits treated by autografting, homografts also were applied a few month after-

wards. All the skin homografts died in the first 2 weeks after grafting. The autografts survived and were kept under observation for more than 6 months.

Observations on the process of survival of skin grafts showed that the vascular changes arising at first in auto- and homografts are very similar. For the first 2-3 days after operation, the grafts were lacking in circulation and were pale in color. The vessels were dilated and had lost their clear outlines. Pink areas appeared in the grafts 3-4 days after operation. Capillaroscopy revealed that the dilated vessels were full of stationary blood (Fig. 1). In places in the vessels of the auto- and homografts slow movement of blood could already be seen. Evidently at this time, vessels had grown in the receptor area and had begun to form anastomoses with vessels in the graft. After 5-6 days the whole graft had become rose pink or red in color. The vessels were filled with blood. Movement of blood could be seen in them.

Beginning on the 6th day after transplantation, disturbances of the circulation in the homografts began to appear. The color of the graft changed in 24 hours from pink or red to dark blue or even black. The blood flow ceased. On capillaroscopy hemorrhages were seen along the course of the vessels (Fig. 1,b). Evidently in the vessels of the homograft, thrombosis has begun to occur, accompanied by rupture of the walls of the vessels or disturbances of their permeability leading to the formation of multiple hemorrhages.

Further changes in the homografts comprised drying and conversion into a scab, under which old blood gathered and epithelialization of the wound proceeded. At the end of the first or middle of the second month after transplantation, the wound margins had drawn together, and a scar had formed in place of the skin defect. In two rabbits (Nos. 786 and 77) the process of survival and death of the homografts proceeded somewhat differently. On the development of hyperemia in the vessels of the homografts, thrombosis did not develop. Their color became less clear. Simultaneously on the 10-12th day after transplantation, the epithelia of the homografts became intensely desquamating. The grafts became covered with scabs consisting of desquamating scales. When the scabs were removed at first, the surface of the graft beneath was pink in color, but later ulceration of the epithelium was observed. The vessels of the homograft could not then be distinguished. In the two cases described we regarded this as the moment of death of the homografts. Later, the homografts dried and were converted into scabs.

A comparative analysis made with autotransplantation of skin grafts shows that after 6-9 days their color was pale. The vessels acquired a clearer outline (see Fig. 1). From 10-12 hours after transplantation the autografts can hardly be distinguished from the surrounding skin. Sometimes slight desquamation of the epithelium from the grafts is observed.

Serological examination of the recipient rabbits with homografts showed that in 19 out of 28 of them antibodies were formed. In one recipient not showing the presence of antibodies, on examination of the serum by means of the ordinary hemagglutination reaction it was possible to detect incomplete antibodies. For the examination of this serum for incomplete or monovalent antibodies a standard weight of erythrocytes was prepared not in physiological saline but in a 20% solution of human serum albumin.

In none of the 13 rabbits with autotransplanted skin grafts were antibodies found.

Comparing the changes in antibody development with the character of the vascular changes arising in the skin grafts, a correlation is shown between the antibodies appearing in the serum of the recipient and disturbances of the circulation in the homograft. By way of illustration we quote data for rabbit recipient No. 1236 (Figure 2).

Until the 7th day after homoplastic grafting the serum of the recipient contained no antibodies. Commencing on the 5th day there were hemorrhages in the graft. On the 7th day after grafting, antibodies were found in a titer of 1 : 4. Disturbance of the circulation in the homograft corresponded to the time of discovery of antibodies, showing itself on the 7th day after operation also.

Similar results were obtained on examination of the majority of the other rabbit recipients. These results are given in the table.

From the results given it is clear that the disturbance of circulation or the start of death of the homografts occurs as a rule on the 6th-9th day after transplantation, and in the majority of cases this corresponds

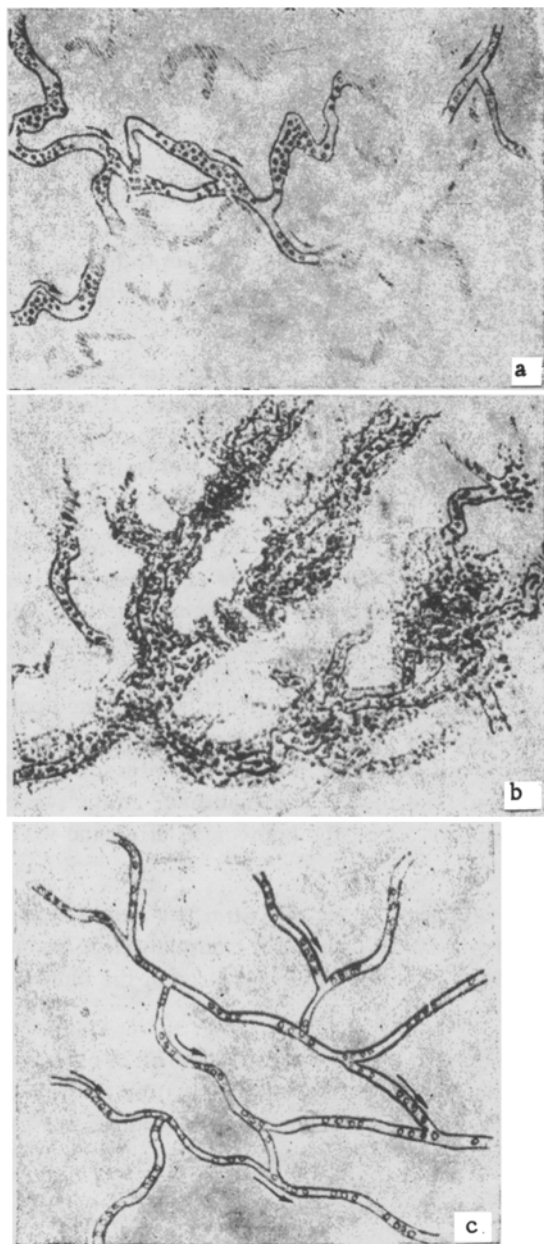


Fig. 1. Progress of the vascular changes in auto- and homotransplanted skin grafts in rabbits. a) vessels in the graft 3-4 days after transplantation. The capillaries are dilated and filled with blood. In places there is slow movement of blood; b) vessels in homograft 6-9 days after transplantation. Hemorrhages are seen along the course of the vessels; c) vessels in the autograft 6-9 days after transplantation. The capillaries are filled with flowing blood.

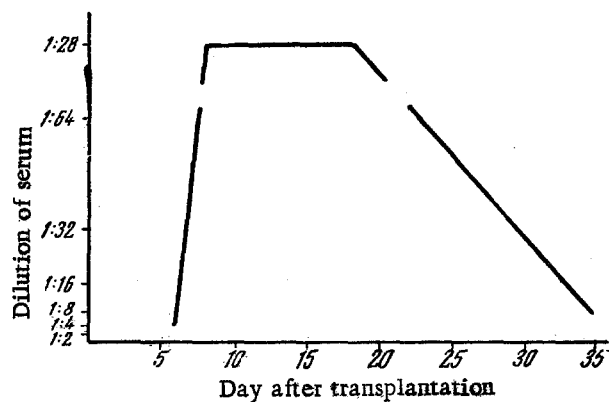


Fig. 2. Changes in the antibody titer.

to the time of discovery or of increase in the amount of antibodies in the serum of the recipient. Only in two recipient rabbits Nos. 786 and 77 were antibodies found on the 5th-9th day after transplantation, while death of the homograft ensued at the end of the second week. The cause of death of the homografts in rabbits Nos. 786 and 77 is not clear. It may be mentioned, however, that in relation to the immune serum of various recipient rabbits, the erythrocytes of recipient rabbits Nos. 786 and 77 were very similar to the erythrocytes of their donors.

Thus in the serum of the majority of recipient rabbits, immune antibodies appeared as a result of homoplastic skin grafting. In 8 recipients, no antibody could be detected by the method which we were using. It is possible that it was present in small quantity or in a state of incomplete antibody.

In support of this idea may be quoted the results of a number of authorities who found that incomplete antibodies appeared in the blood of mice and dogs after homoplastic grafting of skin and kidney (Muirhead and Groves). The absence of antibody production in response to the homografted tissue could also be a result of the lowered immunological reactivity of the rabbits to produce antibodies. We must bear in mind that tissue incompatibility, like any other immunological condition, is determined not only by humoral but also by cellular factors.

From the results mentioned above it is clear that survival of auto- and homografts of skin in the first few days after transplantation was very similar. Antibodies appeared in response to homografted tissue and not to autografted tissue, and moreover the onset of death of the homografts corresponded in time to the discovery of antibodies. This all suggests that antibodies are the

response of the recipient to the antigenically foreign tissues of the donor. In work on immunological relationships, M. R. Gashek observed during successful homografting of skin between partners in embryonic parabiosis, loss of

Morphological and Humoral Changes arising with Homoplastic Skin Grafting in Rabbits

Identification No. of Recipient Rabbit Identification No. of Donor Rabbit	Appearance of hemagglutinins — recipient serum (days after transplant)	Disturbance of circulation signs of homograft death (days after transplant)
1236/1717	6	6
71/811	7	7
812/813	5	6
177/829	7	7
510/46	6	7
811/71	7	6
818/407	9	Between 5th and 9th
77/14	9	11—15
786/407	5	9—15
442/407	Between 7th and 8th	Between 7th and 8th
407/818	Before 8th	7
1717/1236	Between 9th and 12th	6
292/603	6	7
643/701	Between 8th and 18th	8
701/643	Before 14th	8
365/333	6	Before 10th
333/365	5	Before 10th
756/786	6	9
866/67	Between 8th and 9th	Between 9th and 10th
67/670	Antibodies not found	7
936/71	» » »	7
14/77	» » »	7
46/813	» » »	7
813/46	» » »	8
603/292	» » »	Between 8th and 9th
829/177	» » »	Between 7th and 8th
235/333	» » »	6
363/786	» » »	9

the power of the recipient to produce antibodies (hemagglutinins) against the donor's erythrocytes.

In addition the extensive experience of bacteriology and also noninfective immunology, in particular blood transfusion, which is essentially a homotransplantation of fluid tissue, shows that antibodies arising in the body in response to the introduction of a foreign antigen always lead to destruction or rapid removal of the antigen.

This may serve only to prove that antibodies arising as a result of homotransplantation of skin are also an indication of the reaction of the recipient directed towards removal of the foreign antigen and preservation of the normal internal environment of the body.

#### SUMMARY

Antibodies were found in 19 out of 28 rabbit recipients which were examined following homoplastic transplantation of the skin. There were no antibodies found in 13 rabbits with autotransplanted flaps of skin. With the aid of capillaroscopy it was established that disturbance of circulation in the homotransplantate corresponded to the time when the antibodies were found in the serum of the recipient.

#### LITERATURE CITED

- [1] M. R. Gashek, Czechoslovak Biol., 2, 5, 267-282 (1953).
- [2] M. R. Gashek, Czechoslovak Biol., 3, 6, 344-350 (1954).
- [3] N. N. Elansky, New Surgical Records, 3, 11, 597-603 (1923).
- [4] E. A. Zotikov, Byull. Eksptl. Biol. i Med., 7, 58-60 (1956).\*
- [5] I. Khutnaya, Folia Biologica, 2, 5, 284-295 (1956).
- [6] D. B. Amos, P. A. Gorer, B. M. Mikulska, R. E. Billingham, E. M. Sparrow, The Brit. Journ. of Experim. Pathol., 35, 2, 203-208 (1954).
- [7] L. M. Converse and F. T. Rapoport, Annals of Surgery, 143, 306-315 (1956).
- [8] D. A. Darcy, Nature, 163, 98-100 (1949).
- [9] P. A. Gorer, Ann. N. Y. Acad. Sci., 59, 365-373 (1955).
- [10] L. W. Lehrfeld and A. C. Taylor, Plastic and Reconstructive Surgery, 12, 6, 425-431 (1953).
- [11] E. F. Muirhead and M. Groves, Archives of Pathology, 59, 223-231 (1955).
- [12] R. I. Scothorne and I. A. McGregor, Journ. of Anatomy, 87, 379-386 (1953).
- [13] M. Simonsen, I. Buemann, Acta Pathol. Microbiol. Scandinav., 32, 1, 1-84 (1953).

---

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