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THE MICROCIRCULATION IN RAT SKIN ALLOGRAFT DURING REJECTION AND TREATMENT WITH CORTISONE AND TRASYLOL®

By

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INTRODUCTION

Most previous studies of the vascular changes during immunological rejection of skin allografts have been histological. *Medawar* (12) and *Scothorne & McGregor* (19) studied rabbits, *Rolle et al.* (15) and *Wiener et al.* (24) mice, *Waksmann* (23) rats and *Henry et al.* (7) man. They all demonstrated that, after a period of healing, progressive disintegration of the vascular system took place. The vascular walls were invaded and destroyed by lymphocytes which sometimes obliterated the vascular lumen. At last, perivascular haemorrhage was followed by necrosis of the grafts.

A few vital microscopic studies of these phenomena have been carried out by *Taylor & Lehrfeld* (20) on rats, by *Rolle et al.* (15) on mice and by *Converse & Rapaport* (4) on man. They all used vital stereo-microscopic methods, and showed that, after re-establishment of the circulation beginning two days after transplantation, a progressive vasodilatation, stasis and extravasation occurred from the fourth or fifth day after operation.

This process is thought to be mediated by pharmacologically active substances liberated by the contact between sen-

sitized lymphocytes and the specific antigen (16). Among other substances the vasodilating plasmakinins have been the object of interest, although very little is known of their function (2).

Corticosteroids are known to considerably prolong the survival time of allografts. A prolongation by 3 or 4 times has been found in rabbits (1,18) and by 50 per cent in mice (13). The vascular changes in allograft rejection in mice during cortisone administration were studied by *Rolle et al.* (15) who found no morphological difference from the control grafts.

Studies of the microcirculatory changes in rat skin allografts and the possible influence of the anti-kallikrein- and thereby anti-plasmakinin agent, trasylol®, and of cortisone are reported below.

METHOD AND MATERIAL

An Ortholux Leitz microscope with an Ultropak objective was used as described by *Marckmann* (10). This method permits the use of incident reflected light which atraumatically visualizes capillaries, arterioles and venules to a depth of about 60 μ m. The inevitable loss of light was compensated by electronic transmission to a tv monitor. In this way a magnification of about 500 $\times$ was obtained. The picture was transmitted to a videorecorder, rendering repeated observation possible. Furthermore, it was possible to take single photographs and motion pictures (3).

The experiments were performed using two different rat strains, *i.e.* Wistar and Shermann rats to ensure rejection of the allografts. 16 allografts and 16 control autografts were obtained from 8 pairs representing both rat strains. From both flanks a circular piece of skin, 20 mm in diameter was excised. The right-sided grafts were exchanged, and the left-sided immediately repositioned, both after removal of panniculus adiposus and carnosus. The operations were performed in intraperitoneal Nembutal® anaesthesia, if necessary supplied with ether inhalation. Before operation the hairs were removed by shaver and barium sulphide paste.

Beginning post operation and continuing till necrosis, 42 observations were recorded. Comparison was made between the microcirculation of allografts, autografts and normal skin in order to establish the time of rejection. The parameters were vasodilatation, vasomotion, flow-velocity and extravasation.

In the experiments with trasylol, which is an antagonist of the plasmakinin precursor kallikrein (5), the same procedure was used on 11 animals. Intra-

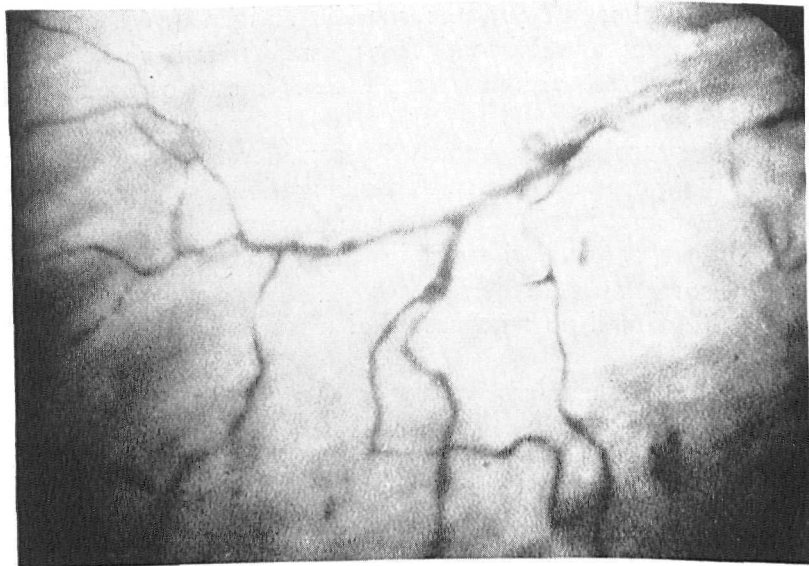


Fig. 1.

Normal rat skin. Normally calibrated vessels are seen to empty blood into the vein in the center of the picture.

peritoneal injections of trasylol were started on the day of operation. Three thousand i.u. per kg. were given every second hour until rejection. 36 observations were recorded.

The experiments with cortisone were performed in the same way. Cortisone was injected intramuscularly in doses of 10 mg per kg twice a day starting on the day of operation till rejection. In this way 43 observations were recorded from 14 animals.

RESULTS

1. Untreated Allografts

The revascularisation process was identical in the allo and autografts during the first 4 days after operation. The first and second day a slow filling of the vessels took place, but no circulation. From the 3rd day a slow circulation could be seen. The vessels were slightly dilated. Vasomotion was not observed.

From the 4th day the number of visible vessels was the

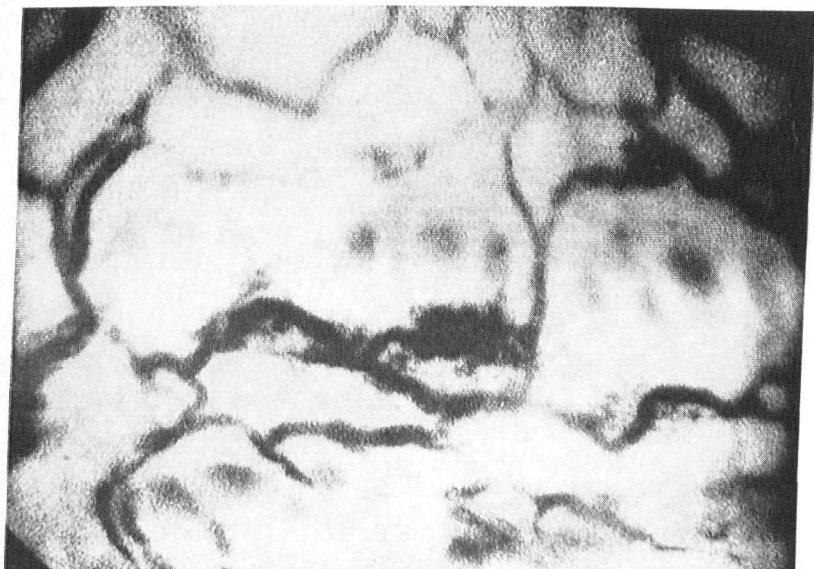


Fig. 2.

Seven day-old allograft. Perivascular extravasates are seen around dilated vessels with indistinctly demonstrated walls.

same as in intact skin, and all of them seemed to be involved in circulation. The circulation velocity, however, was slower. All vessels were slightly dilated and little vasomotion was noted. From the 5th day a difference in the circulation pattern between allo- and autografts could be seen. The circulation in the autografts became normal, as described by *Marckmann* (11). From the 5th day until necrosis occurred in the allografts (about 9 days after operation) a progressive disintegration of the circulation took place. Vasodilatation was most pronounced in the venules and local ectasies appeared. The flow velocity and the vasomotion decreased. From the 6th day the outlines of the vessels became blurred and surrounding extravasates became visible. From the 8th day the circulation almost stopped, and extravasates were seen in large areas of the grafts. From the 9th day most grafts were necrotic.

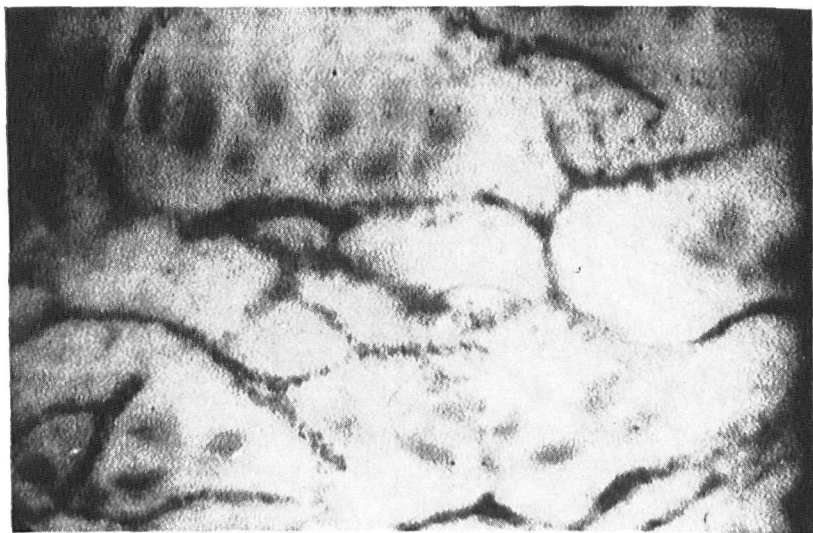


Fig. 3.

Eight day-old allograft. Blurred remnants of the vessels are seen.

The average survival time, from operation to necrosis, was 9.8 days.

2. *Cortisone-Influenced Allografts*

The microcirculatory changes were the same as described above. However, they were delayed by one or two days. The average survival time was 11.1 days.

3. *Trasylol-Influenced Allografts*

The microcirculatory changes did not differ from the spontaneous. The average survival time was 9.7 days.

The difference between the average survival time for the untreated and the cortisone-influenced grafts was statistically significant (< 0.01), whereas the difference between the untreated and the trasylol-influenced grafts was statistically non-significant ($p > 0.5$).

DISCUSSION

As pointed out by *Marckmann* (11) the method used in this investigation renders it possible to study, with minimal injury to the object, the dynamics of microcirculation. However, it must be kept in mind that it is only the erythrocytes which present the contrast (10) and are visualized. The leucocytes and specially the plasma and the vessel walls, which indicate the true caliber of the vessels, are invisible. This represents a real problem in the microcirculatory studies because considerable areas are occupied by pure plasma as a result of the so-called plasma skimming phenomenon. In spite of this, the method enables one to estimate vasodilatation, vasomotion, flow-velocity and extravasation.

The delayed immune reaction, which seems responsible for the rejection of the allografts, is mediated by lymphocytes invading, destroying and thrombosing the vessels (12, 23, 22, 24). The resulting microcirculatory disintegration described above is analogous to that found in previous studies (20, 15, 4), except for the species-dependant differences in initiation and duration of the process. Like in the papers of *Wiener et al.* (24) and *Waksmann* (23), the process was observed to occur first and most intensive on the venous side of the microcirculation. Similarly, in unspecific inflammation, the venules seem to be the most vulnerable part of the small vessels (9, 25).

As shown in earlier studies on this subject, systematically administered cortisone prolongs the survival time of allografts. However, when started, the process included qualitatively and quantitatively the same steps as observed in untreated grafts, as also demonstrated by *Rolle et al.* (15). In this study the survival time was prolonged one or two days, whereas *Billingham et al.* (1) and *Medawar & Sparrow* (13) using almost the same doses of cortisone, observed a prolongation by a factor 3 or 4. The difference may be explained by the considerable difference in cortisone sensitivity of lymphocytes from various species (17).

Plasmakinin activity has been demonstrated in other types of delayed hypersensitivity, for instance in allergic contact dermatitis of man (14) and guinea pigs (8) and in the tuberculin reaction in man (6). No information about kinin activity in skin allografts is known to the author. In this study trasylol® had no influence on the microcirculatory disturbances and the survival times. However, the absorption from the peritoneal cavity of rats must be considered dubious, although this has been demonstrated in dogs (21).

SUMMARY

By television vital microscopy the microcirculatory changes of rat skin allografts during rejection were studied. The process was accompanied by progressive disintegration of the circulation, extravasation, and vascular destruction. The first changes appeared in the venules. Furthermore, the influence of trasylol® administered intraperitoneally and of cortisone given intramuscularly was studied. Trasylo® did not change the events. Cortisone significantly prolonged the survival time of the grafts, but did not influence the vascular picture.

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