SOME HISTOLOGICAL CHANGES OF THE SKIN IN HOMOGRAFTS AND IN CORTISONE THERAPY APPLIED TO HEALTHY AND IRRADIATED RABBITS

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Only slight attention has been paid to histological changes in skin homografts [3, 7, 12, 13, 14], and very little information is available on their histochemistry. Homografts in radiation sickness form a subject of special interest. One method of prolonging the survival time of the homograft is to use cortisone [11, 15].

EXPERIMENTAL METHOD

Acute radiation sickness was induced in adult rabbits by a single general dose of 600 r (current 18 ma, potential 205 kv, 0.5 mm Cu filter, focusing distance 50 cm, dose rate 21 r/min). A portion of skin 3 cm in diameter was removed from the inner surface of both ears of healthy rabbits, or from the ears of irradiated animals at various times after exposure. The wound was immediately covered with a graft cut from the outer surface of an ear and preserved for four days at 4-6° in V. I. Popov's preservative fluid [8]. All the animals received daily intramuscular injections of 1.5 mg/kg of cortisone; the irradiated animals received the cortisone as well as therapeutic treatment for acute radiation sickness. Biopsy speciments taken up to 42 days after homografting were studied histologically.

EXPERIMENTAL RESULTS

Observations were made on 115 cutaneous homografts made on 60 rabbits. The best results were obtained with the skin homografts made onto healthy animals treated with cortisone: the graft remained soft and elastic for 25-30 days. Without the cortisone therapy, the transplants took for only up to seven days (Fig. 1). In irradiated animals the use of cortisone enabled the cutaneous homograft to take at all periods of the acute radiation sickness. The grafts remained viable for 35-45 days.

In the non-irradiated animals, 24 hr after homografting there were no changes in the appearance of the grafts. Nevertheless in the fragment numerous pseudoeosinophilic leucocytes and erythrocytes could be seen. There were spaces at the edge of the graft between it and the surrounding skin. For the whole of its extent the bed of the graft consisted of the fibrous connective tissue of the perichondrium above which blood depots were sometimes to be found.

By the end of the third day the cytoplasm of the epithelial cells of the graft had become more basophilic through an increase in the amount of ribonucleoproteins, and mitotic figures could be found. On the other hand, the epidermis of the graft appeared dystrophic, and in parts was infiltrated with pseudoeosinophilic leucocytes; the cytoplasm of the cells of all the layers was oxyphil. In parts the epithelium was completely necrosed, while small scabs developed on the surfaces of the abscesses which had formed. The vessels of the graft were filled with blood cells of normal appearance. The peripheral portions had necrosed at the points which had come in contact at grafting with the surrounding skin. The host ear developed a stratified epidermis which increased in thickness, differentiated, and grew out along the granulation tissue. The cytoplasm of the mitotically dividing cells of the deep zone of the regenerating tissue was considerably richer in ribonucleoproteins than was the proliferating epithelium of the homograft growths. Unlike the latter, the cells of the middle zone of the regenerating portion developed from the epithelium of the recipient were always rich in grains of glycogen.

Seven – ten days after grafting, the whole of the epidermis of the transplant necrosed and fell away, and in its place there was a thin layer of cells formed by the epithelium of the hair follicles. It consisted of 1-2 layers of thickened cells having a basophil cytoplasm and dividing mitotically. In some regions there were scabs and small

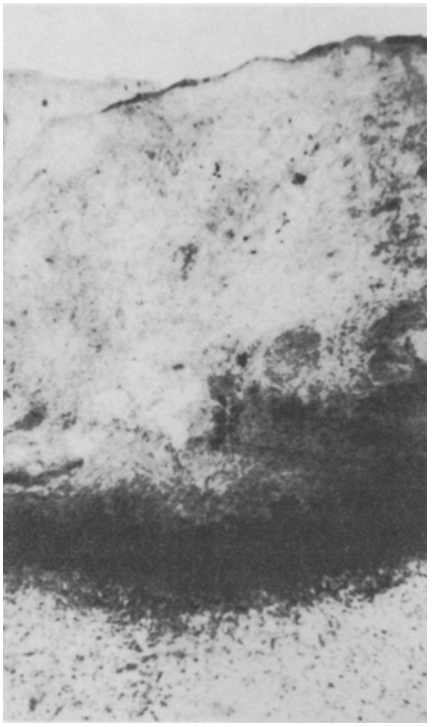

Fig. 1. Portion of a necrosed homograft on the 20th day after transplantation without cortisone or irradiation. A demarcation zone can be seen between the homograft and the granulation tissue at the junction. Formalin, hematoxylin-eosin. Objective 3.5 ×, ocular 20 ×.

abscesses. In the peripheral portions of the graft and in the underlying regions of the bed there was a gangrenous inflammation.

Subsequent events in the graft took a normal course: over a period of many days there were profound dystrophic and necrotic changes as well as a cellular proliferation. However by about the 20th-25th day the superficial part of the graft had become transformed completely into a scab. Only in a few parts of the surface of the dermis was there a thin layer of epithelium, containing less ribonucleoprotein than was present in the regenerating epidermis of the host, and containing glycogen in those areas which were proliferating most actively. The deep parts of the graft were retained for 30-35 days. Granulation tissue grew between the collagenous bundles. Emerging from the junction layer, which had thickened at this time, cords of connective tissue and of the capillary endothelium pushed aside the thick collagenous bundles of the graft. For a long time the latter could be found between the newly formed cells. As time passed, in the rabbits, just as had been found in piglets [2], the intercellular substance of the graft was gradually absorbed.

In the latent period of acute radiation sickness the changes in the first days after homografting were mainly the same as in the non-irradiated animals. At a time 1-3 wk after transplantation there was more proliferation than dystrophy throughout the fragment. The epithelial cells divided mitotically, their cytoplasm was rich in ribonucleoprotein, though it is true this effect was not so marked as it was in the host cells. Granulation tissue formed in the junction layer and in the deep part of the fragment, and there were hemorrhages into it. At all stages studied the fragments of skin remained viable (Fig. 2), a result which is in agreement with clinical observations [9].

The dystrophic changes of the fragment at the height of radiation sickness occurred earlier than when transplantation was made in the latent period, and the proliferative processes in the skin surrounding the fragment were suppressed: the epidermis regenerated slowly through the formation of new granulation tissue in which the first stages of development were held back. Nevertheless mitoses could be observed in the connective tissue of the host. Between the fragment and the bed on which it lay and in the homograft itself hemorrhages always developed. The sloughing off of the fragment from the bed was probably due to the slowing down of the production of granulation tissue in the junction layer. In some animals, even by the 7-12th day there was an increased infiltration into the fragment of pseudoeosinophil leucocytes, and a broad demarcation band was formed which separated the dead surface of the fragment from the viable portion beneath. In these rabbits, by the end of the second week the cells of the fragment had died and had formed a scab beneath which the epithelium of the surrounding skin gradually grew.

When the skin was transplanted during recovery from acute radiation sickness the tissues of the homograft remained viable (Fig. 3) for a longer time than when homografting was carried out at the height of the disease, but for not so long as when the operation was made during the latent period. Dystrophic changes in the epidermis of the homograft were observed by the end of the second week, though in some parts viable epithelium could be observed as late as the 35-40th day. In other cases the superficial portion of the dermis and epidermis changed into a scab, and the fibrous intercellular substance of the deep portion of the reticular layer of the fragment served as the basis for the outgrowth of new host granulation tissue. Its epidermis proliferated very vigorously when the graft was made during recovery from acute radiation sickness, and it moved beneath the necrosed portions of the fragment which were sloughing off. No matter in what period of the disease a cutaneous homograft was made, we never observed such extensive gangrenous processes as occurred in the non-irradiated group.

In the skin homografts dystrophic, necrotic and proliferative changes succeed one another, but subsequently the latter at first preponderate, and then cease altogether. The effect of cortisone on the tissues of the fragment is to prolong viability; without cortisone the homografts perish by the 12-15th day [1]. The most viable are the fragments of

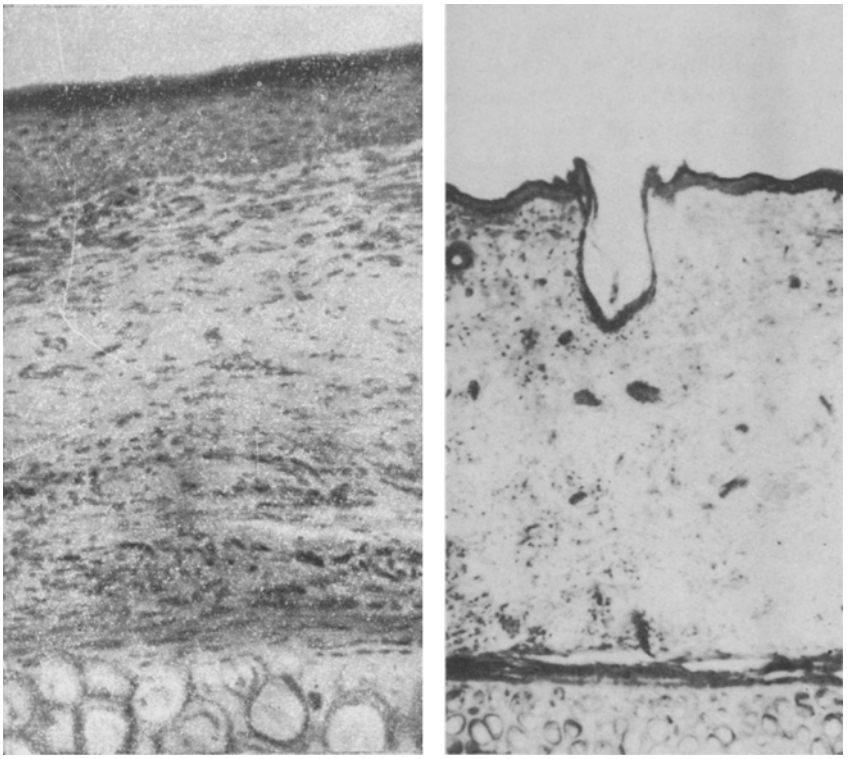

Fig. 2. Part of the homograft on the 40th day after transplantation during the latent period of acute radiation sickness; action of cortisone. Epidermis and dermis appear viable. Formalin, hematoxylin-eosin. Objective 9 ×, ocular 20 ×.

Fig. 3. Portion of homograft, still appearing viable; 20th day after transplantation during recovery from acute radiation sickness; cortisone used. Formalin, hematoxylin-eosin. Objective 3.5 ×, ocular 20 ×.

skin transplanted in the latent period of radiation sickness. This result agrees with what was found for autografts made onto burns in irradiated piglets [6].

Most probably the suppression by radiation and especially by cortisone of the reactive changes in the body [4, 5] is the reason for the reduced immune reactions of the host to the homograft. The fact that the fragment perished more rapidly when the graft was made during the recovery period, and still more so when it was made at the height of the disease can be explained entirely in terms of the massive hemorrhages always observed in the bed beneath the fragment. We must note that even at the height of the disease regeneration of epithelium and connective tissue was never completely suppressed, but the rate of the regenerative processes was reduced, and this finding corresponds with current information [10].

The proliferative processes in the epithelium of the homograft and the surrounding host skin differ both histochemically, in a number of ways (accumulation of glycogen, increase of ribonucleoprotein), and with regard to differentiation of the graft. The epithelium of the homograft scarcely ever contains glycogen, it is poor in ribonucleoprotein; the newly-formed layer is extremely fine and there is no differentiation in depth. By contrast, the epithelium of the host consists of a stratified layer gradually becoming cornified; the cells of the deep zone are rich in ribonucleoprotein, while the middle layer is rich in glycogen. All these facts point to the short survival time of the homograft tissue even when there are considerable proliferative changes.

SUMMARY

The skin of 25 rabbit ears was examined 1-42 days after homografts had been made and cortisone treatment given. The grafts were made at various stages of acute radiation sickness. Under the influence of cortisone the homograft tissues remained viable for a longer period. The latent period of acute radiation sickness was the most favorable for homografting. At the peak of the disease regeneration of epithelium and connective tissue is not suppressed, but delayed.

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