POST-TRAUMATIC SKIN REGENERATION IN RATS WITH TRANSPLANTABLE SARCOMA 45

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At the present time, there is no single opinion regarding the relationships between regeneration and the growth of tumors. Certain authors [3,5,6,7] have mentioned the possibility of a transition from the regenerative process to that of cancerous growth, whereas other authors consider these processes antagonistic and mutually exclusive.

The aim of the present work was to undertake a histological study of the general and local effect of a neoplasm on the regeneration of skin, under varying locations of the original wound with respect to the position of the tumor, and under varying intervals of time between sarcoma transplantation and skin injury.

EXPERIMENTAL METHODS

The experiments were carried out on 120 white rats (both sexes) weighing 120-150 g. The animals were divided into four groups consisting of 30 animals per group. Animals in the first group received no sarcoma implants and served as controls. The remaining animals all received transplants of sarcoma 45 in the right side at the same time. The control animals were given 3 skin wounds: on the right and left flanks and on the outer surface of the right thigh. The experimental animals of the second, third, and fourth groups received four skin wounds on the 8th, 15th, and 22nd days, respectively, after transplantation. These wounds were made: above the tumor nodule; at a distance of 1.5-2.0 cm from the margin of the sarcoma; on the opposite side of the animal; on the external surface of the right thigh. The wounds were inflicted with a skin-trepan and involved all the cutaneous layers; they were of circular outline (diameter 8 mm) and received no dressings but were allowed to heal under the scab. They were measured from the sixth day onwards until healing was complete. This was accomplished by drawing their outlines on a cellophane film, followed by use of a planimeter and statistical treatment of the results. Groups of animals were killed at intervals of 1,2,4,5,12, and 16 days after wounding. The areas of wounding were excised and fixed in Zenker's fluid. Material was embedded in celloidin-paraffin and the sections obtained from it were stained in hematoxylin-eosin, picrofuchsin, by Weigert's method for fibrin, and silver impregnated.

EXPERIMENTAL RESULTS

Of the 44 wounds inflicted above the tumors only 6 or 13.7% of the total healed successfully (see table), taking 11.3 days to do so; the rest developed into abscesses. The wounds situated near the tumors, in animals which had been operated on 8 and 15 days after implantation, healed more rapidly than the wounds on the right flank of the control animals ($P < 0.02$ and $P < 0.001$). The healing of wounds on the left flank and right thigh suffered a significant retardation of healing in those animals operated on 22 days after tumor implantation ($P < 0.02$ and $P < 0.03$).

Healing of wounds in controls and those situated far from implanted tumors. Twenty-four h after the operation, the wounds in the control animals were covered by a leucocyte-necrotic scab. The floor of the wound was formed from subcutaneous cells showing signs of conversion to granulation tissue. On the second day, the edge of the epidermis showed the greatest amount of regeneration, spreading over the granulation tissue under the scab. By the same time, in animals which had received sarcoma implants (and wounds inflicted 22 days after implantation), practically

Effect of Sarcoma 45 on Period of Wound Healing (in days)

Group of animals	Site of wound				
	above tumor	on right side: without tumor	on right side: near tumor	on left side	on thigh
1st (control)	—	11,8	—	12,2	11,2
2nd (experimental)	3 wounds out of 14 healed	—	9,9	12,6	11,6
3rd »	1 wound out of 15 healed	—	9,6	12,3	12,0
4th »	2 wounds out of 15 healed	—	11,9	13,6	12,8

no regeneration had taken place and the whole of the wound base was occupied by clotted tissue fluid containing a network of fibrin and leucocytes, far exceeding that in the controls. On the 4th day, the regenerated epidermis in the controls was large and distributed over the completely formed granulation tissue, which consisted of a horizontal layer of fibroblasts with vertical vessels.

In the experimental animals, the amount of regeneration was very small and the extremities of the regenerated tissue were destroyed by leucocytes. On the eighth day, the regenerated epidermis sometimes formed "a wall" [1], propping up the granulation tissue, which in this case was characterized by a large number of leucocytes. By the 12th day, the epithelialization of the wound was complete in the control animals but not in the group of experimental animals. A more intense accumulation of matric substance, but a decreased formation of both argyrophilous and collagen fibers, could be noted in the experimental animals as compared with the controls at this time.

Healing of wounds near tumors. The skin near the site of the sarcoma (over a distance of about 1 cm from it) appeared to have experienced some stimulation. Thus, instead of the usual 2-3 layers of epidermis exhibiting mitotic division, 5-6 cell layers were involved in this activity and the rudimentary hair follicles underwent further growth. Formation of granulation tissue and regeneration of the epidermis had already become established by the second day after the wounding and by this time the granulation tissue was, in fact, visible as a definite proliferation of cellular elements resulting from both mitotic and amitotic divisions of young fibroblasts. On the fourth day, the regenerated epidermis was extensive, and by the eighth day, had almost covered the whole wound. The latter was completely epithelialized by the twelfth day although the maturation of the granulation tissue and hair formation had suffered some retardation. Many of the large fibroblasts persisted until the 16th day of wound healing and, by that time, the collagen bundles were not completely formed.

Condition of wound above tumor. We consider that three variants exist with respect to the condition of the wound above the sarcoma. These variants depend on the amount of growth which the cancerous tissue has made towards the skin and this, in turn, usually depends on the period of time which has elapsed since implantation of the sarcoma.

Variant 1. The sarcoma, in this variant, has penetrated deeply into the subcutaneous tissue. The base of the wound, above the tumor, consists of a massive layer of subcutaneous tissue and even after one day this layer shows signs of conversion into granulation tissue. The process whereby granulation tissue and regenerated epidermis is formed in these wounds is the same as that in controls and by the 12th day the wounds above deep-seated sarcomas have healed. Mature granulation tissue can be seen to have grown over the cells of the sarcoma in such cases (c.f. Fig. 1).

Variant 2. The sarcoma cells have begun to grow into the dermis. In such cases, the processes of granulation tissue formation, epidermal regeneration and growth of the tumor at the base of the wound go on simultaneously. After one day, the wound is covered over with a scab. Between it and the tumor there is formed a layer of subcutaneous cells, narrow in the center but broad at the edge, in which characteristics of granulation tissue appear on the second day. The tumor cells, in all cases, actively proliferate among the cells forming the floor of the wound and among the newly formed granulation tissue cells. The regenerated epidermis appears on the second day. By the

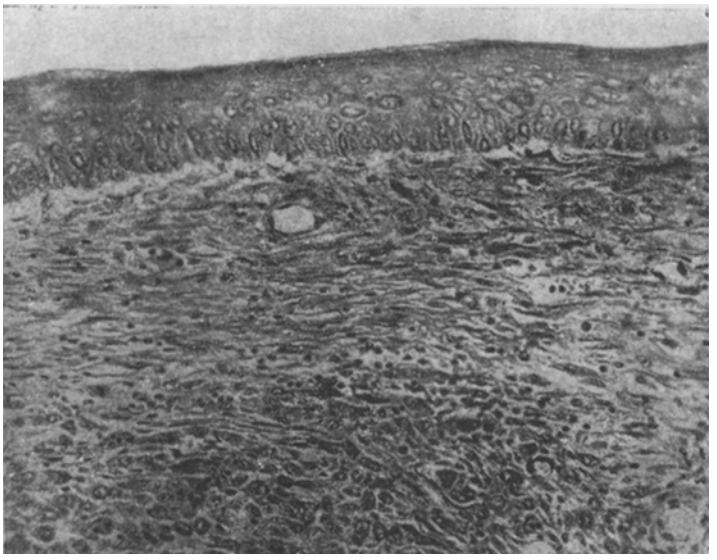

Fig. 1. Regenerated skin above sarcoma 12 days after wounding. Stained hematoxylin-eosin. Ob. × 20, oc. gomal II.

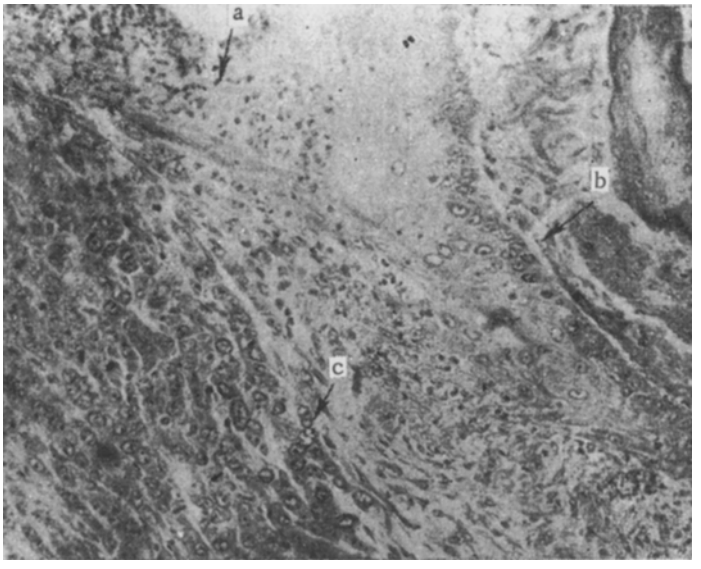

Fig. 2. Margin of wound on fourth day after wounding in a rat with a superficial sarcoma: a) dying regenerated epidermis above sarcoma; b) regenerated epidermis growing along the edge of the sarcoma; c) sarcoma.

fourth day, the regenerated epidermal mass is extensive and mitotic divisions are frequent. The granulation tissue consists of a layer of horizontal fibroblasts with vertical vessels. By the eighth day, the sarcoma has usually grown through the granulation tissue to the scab and necrosis has set in. By the twelfth day, only a narrow strip of granulation tissue remains, and infiltration by the sarcoma cells is almost complete. Sometimes the sarcoma may grow in the old skin. Whenever the sarcoma grows beneath newly formed epidermis separating it from the connective tissue, the former tissue begins to die. The central portions of the sarcoma mass undergo necrosis at this time, the scab liquifies and the wound is converted into an abscess.

Variant 3. When the wound is established on the 22nd day after tumor implantation, the greater part of the dermis has usually undergone infiltration by the sarcoma cells. In such cases, the sarcoma constitutes the floor of the

wound and granulation tissue develops in the narrow interstice between the dermis and the tumor. A wedge-shaped mass of regenerated epidermis is formed on the second day after wounding and, by the fourth day, it has grown into a considerable size. Part of the epidermal mass occurs within the sarcoma tissue and here some cornification and pycnosis of the cells is visible; the other part of the epidermal mass lies between the dermis and the tumor (Fig. 2). By the eighth day, nothing remains of either the granulation tissue or the regenerated epidermis and the wound has become converted into an abscess.

It can be seen, therefore, that the rate of healing of cutaneous wounds in animals with neoplasms depends on the situation of the wound in relation to the neoplasm, itself, and also on the extent to which the tissue of the latter has penetrated into the dermis. Wounds, inflicted on the eighth and fifteenth day after implantation of the sarcoma, show a tendency to delayed healing and the retardation is even greater in the case of wounds inflicted on the twenty-second day. This can obviously be explained in terms of a general change in physiological state of the animal in response to the growth of the sarcoma [13]. Our investigations of the skin in the vicinity of the tumor have shown it to be in a state of stimulation over a distance of approximately 1 cm around the tumor itself; this may be due to some growth substance emanating from the tumor [2,8,12]. Wounds inflicted on the skin in this zone of stimulation heal more quickly than in the controls. These results are in agreement with the finding that, in tissue subjected to stimulation, the processes of regeneration proceed more rapidly [9].

Our findings are in conflict with the opinion of B. P. Tonkin and his associates [10,11], who emphasize that no signs of regeneration can be found in the skin above carcinomas. The process of regeneration is obviously dependent on how far the carcinoma is from the wounded skin. If the carcinoma lies below the skin and its growth has only just commenced, then the cutaneous wound may heal more rapidly than in the controls. With further growth of the carcinoma, such that it forms the whole of the floor of the wound, epidermal regeneration still takes place, but the regenerated cell mass fails to survive. It would seem, therefore, that the relationship between the process of regeneration and of tumor growth is not always characterized by antagonism.

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