EXPERIMENTAL BIOLOGY

HISTOLOGICAL CHANGES IN THE SKIN OF RABBITS FOLLOWING TRAUMA

N. G. Khlopin, N. A. Shevchenko, and L. B. Berlin

From the S. M. Kirov Military Medical Academy, Order of Lenin

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This paper is devoted to a study of histological changes in the skin of rabbits and of the mutual relations of its tissues following localized trauma of the skin and interference with its innervation.

EXPERIMENTAL METHODS

The experiments were performed on 70 rabbits, which were given subcutaneous injections of 1 ml of sunflower seed oil containing formaldehyde ("formalin oil"), or subcutaneous implants of celloidin shavings, under the skin of the back or the thigh. After 3 days in some experiments, and 7 days in others, a piece of skin 1 × 1 cm in areas was excised from over the site of the focus of aseptic inflammation which had developed. Material was also taken at various times after the operation, from 3 hours to 8 months.

Histological changes due to interference with innervation were studied in the skin of the thigh, foreleg, and the back of the foot and heel of both hind legs following excision of a section of the right sciatic nerve 1 cm long at the point where it divides into medial and lateral branches in the thigh; 29 animals were taken for these experiments. Flaccid paralysis of the limb followed immediately after the operation. Skin samples were taken at various times after the operation, from 1 day to 6 months.

The pieces of skin were fixed in Zencker formol or in neutral 15% formaldehyde; in some cases they were embedded in celloidin or celloidin-paraffin, by Carnois' procedure. The sections were stained with Heidenhain's iron hematoxylin, with hematoxylin-eosin, with hematoxylin-picrofuchsin, with "azan" mixture, with azure II-eosin, in some cases by Foot's method for argyrophilic fibers, by the Feulgen reaction, and with Best's carmine stain. Silvering of nerve fibers was done by Lavrentyev's modified method.

We first studied the histology of normal skin taken from various locations, with particular regard to the epidermis. In addition, we followed regenerative processes at the places where the 1 sq. cm. samples of skin had been taken, from the back and thigh.

EXPERIMENTAL RESULTS

The normal epidermis of the back, thigh, and shin of the rabbit consists of 1-2 layers of living cells of the germinative layer, isolated granular elements, not forming a continuous layer, and a cornified squamous layer. The number of cellular layers of the germinative zone of the skin of the heel amounts to 4-5, and at the back of the foot to 6-8, and there is a continuous granular layer. The cornified layer over the heel, which is not covered with hair, is much thicker than at other locations. Free nerve endings are to be found throughout the thickness of the epidermis, including the cornified layer.

The skin surface is more or less smooth, although on the hind limbs projections and papillae of various sizes may be found. The dermis consists of two sharply defined layers, a subepithelial layer containing slender fibers, capillaries, and small nerve ramifications, and a layer containing coarse fibers, hair follicles, and sebaceous glands; sweat glands are absent. Below this is a layer of striated muscle and a subcutaneous cellular layer with infrequent fat cells.

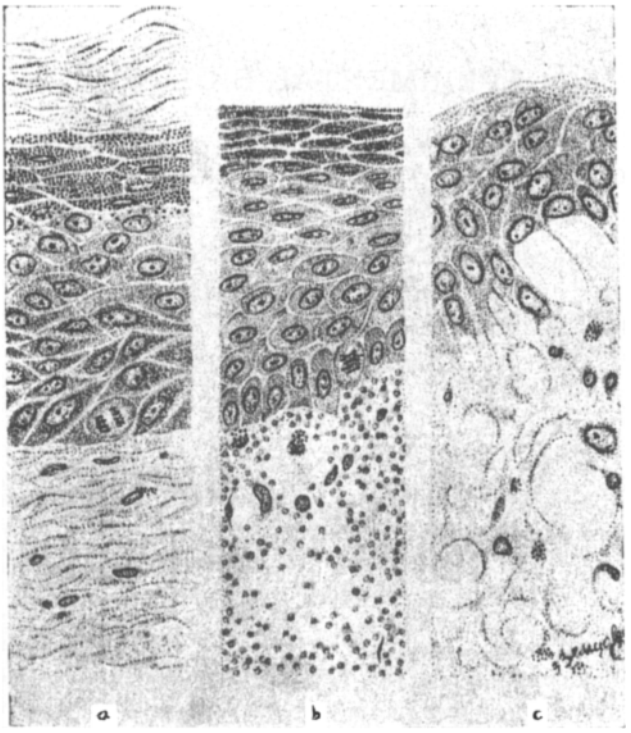

Fig. 1. Various sections of the same epidermal regenerate: (a) on granulation tissue, (b) on blood clot, (c) on edematous connective tissue infiltrated by leucocytes. 15 days after injection of formalin oil, and 8 days after excision of skin. Fixed in Zencker-formol, stained with azure-eosin. Immersion objective 90 ×, ocular 7 ×.

Regeneration of the skin of the back and thigh following excision was followed up to 25 days after the injury. The regenerating epidermis, which is multilayered from the beginning [6], advances gradually under the scab along the granulation tissue which replaces the fibrinous exudate, containing pseudoeosinophilic leucocytes and red cells, which initially filled the wound. In the granulation tissue nitotic division of weakly differentiated fixed cells of the type of fibroblasts is evident, as well as of capillary endothelial cells, and a fine network of argyrophilic fibrils and slender collagen fiber structures appears. Epithelization of the okin defect is usually completed by the 8-9th day. Nitotic activity persists for a long time in the regenerated epithelium, and nucleated cells are present in all its layers, including the uppermost.

Subcutaneous introduction of formalin oil or celloidin shavings caused tissue changes and an inflammatory reaction; these effects were more marked with oil. A connective tissue capsule formed around the oil droplets, containing flattened mononuclear cells and large polynuclear cells, characteristic of oil granulomas [2]. A thick connective tissue capsule gradually formed around the celloidin chips.

A number of changes took place in the epidermis over the locus of inflammation, some of them dystrophic, leading to breakdown of the cells, and other proliferative, leading by enhanced mitotic activity to its thickening and hyperplasia; in some cases ingrowth of epidermis was observed, as has been shown by V. G. Garshin [2], and N. N. Anichkov, K. G. Volkova, and V. G. Garshin [1]. Epithelial hair follicles were also involved in the proliferative process around the granuloma.

We were not able to observe accelerated epilhelization of skin defects when the excision was made 3 days after injection. The regenerating epidermis spread gradually through fibrinous exudate and leucocyte-infiltrated connective tissue, the fixed cells of which displayed enhanced basophilia and mitotic activity.

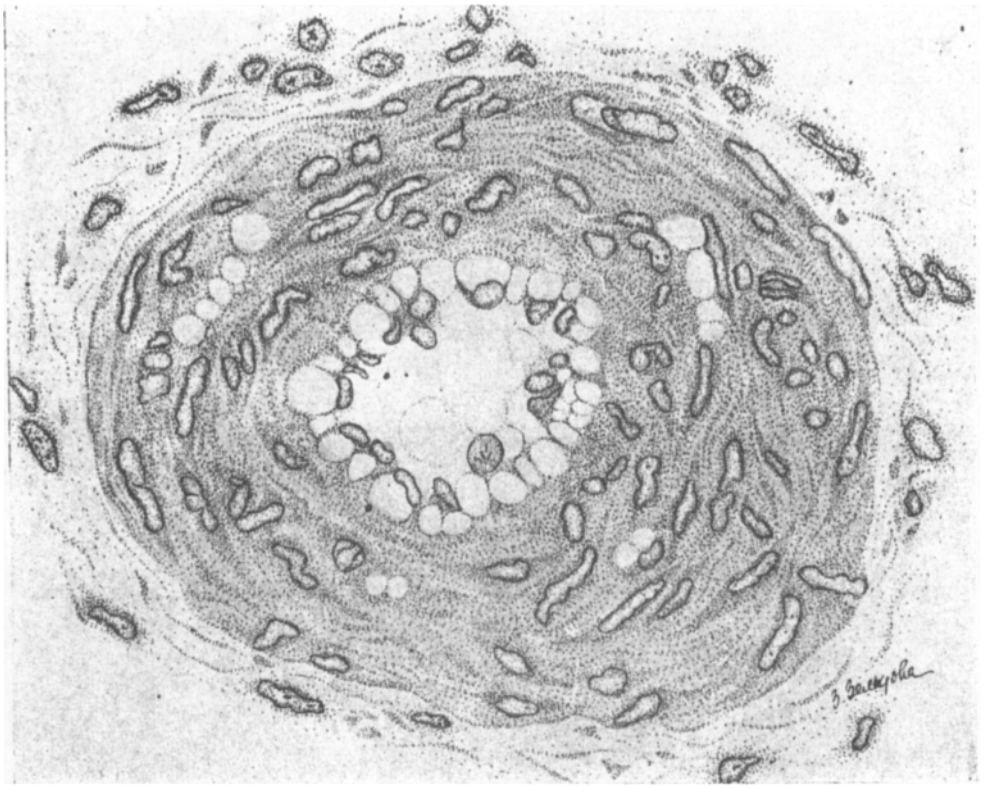

Fig. 2. A small artery with vacuolized endothelium in granulation tissue, from an ulcer in the skin of the heel.
60 days after excision of a sector of the sciatic nerve. Formalin, hematoxylin-picro-fuchsin. Immersion objective 101 ×, ocular Homal 11.

When skin was removed 7 days after introduction of formalin, oil or celloidin proliferative processes were all developed in the inflammatory locus, and the regenerating epidermis advanced through granulation tissue. This led to more rapid epithelization, which was completed by the 6th day in such cases.

Glycogen inclusions, staining with carmine according to Best, were present in the multilayered epithelial regenerate. They were to be found in the perinuclear parts of the cells of the germinative and granular layers, and were no longer visible after treatment of the sections with saliva. Basophilic Feulgen staining nucleoli were visible against the lightly staining background of the contents of the large nuclei. The nucleus of cells of the germinative layer of normal epidermis is smaller and stains more deeply, the deoxyribonucleic acid being distributed diffusely throughout their contents.

It is possible to observe, often in a single section, that the structure of the layer of regenerating epidermis varies according to the nature of its underlying tissue. Epidermis growing over tissue modified by edema and infiltration of leucocytes remains thin, with only sporadic mitoses (Fig. 1,c), whereas that growing over blood clot is somewhat thicker, and is distinguished by the basophilic cytoplasm of its flattened surface cells, which retain their nucleus (Fig. 1,b). Epithelium lying on granulation tissue is particularly thick, and exhibits marked mitotic activity not only in the basal layer, but also in the 2 or 3 layers above this. The cytoplasm of the cells of the germinative layer is basophilic. The stratum granulosum consists of a few layers of cells, and the cornified layer is clearly visible (Fig. 1,a).

The results for animals with damaged nerve supply vary from rabbit to rabbit, and different results are obtained when the nerve is cut at different levels. G. N. Orlova (1954) working on rats, found that development of ulcers depended on the level at which the sciatic nerve was cut.

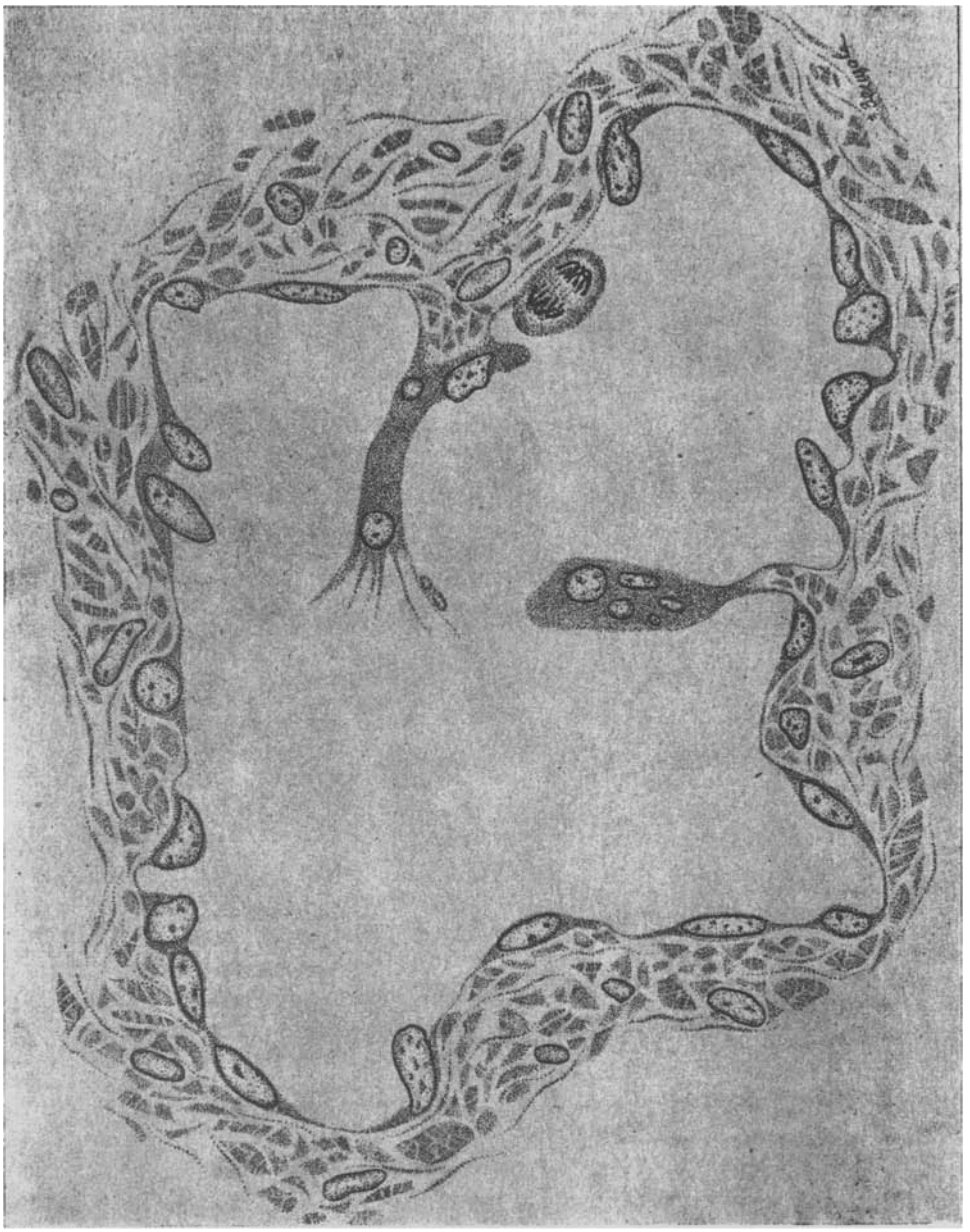

Fig. 3. Intravasal proliferation of a small cutaneous vein in connective tissue in the vicinity of an ulcer on the heel.
60 days after excision of a portion of the sciatic nerve. Zencker-formol, hematoxylin-eosin. Immersion objective 90 ×, ocular 10 ×.

Vasodilatation is seen in the skin of the hind leg of rabbits after section of the sciatic nerve, extending to the capillaries of the papillary zone, as a result of which the connective tissue becomes edematous, and numerous leucocytes make their appearance in it. In some places the epidermal cells increase in volume, the number of layers increases, and the epidermis as a whole becomes much thicker. The basophilicity of the cells of the germinative layer increases, and mitoses appear not only in the basophile layer, but also in the 2 or 3 layers of cells above it. In other parts of the skin of the thigh and shin of the same leg mitoses are absent, and the cells are vacuolized and dystrophic, and somewhat later small necrobiotic foci appear in these areas. This leads after 12-14 days to development of numerous microscopic ulcers in the skin of the thigh and shin, and to

formation of large bleeding ulcers on the heel and back of the foot and paw, which show little tendency towards healing and become infected; the floor of these ulcers consists of necrotic connective tissue infiltrated with leucocytes. These ulcers developed even when the limb was put in a splint and bandaged immediately after the operation, to protect it from trauma during movements of the animal. In some places the thickness of the stratum granulosum increases, and of the cornified layer diminishes. The epidermal cells of some places retain their nucleus in all layers, including the surface one.

At later stages (2-3 months after operation) the skin of the operated leg showed an alternation of dystrophic, necrobiotic, and ulcerated areas with areas of intensified proleferation, in which active spread of thick epidermal tissue is evident, between the cells of which blood cells are to be found. This is associated with repair of previously formed defects, and is accompanied, particularly in the heel region, by inflammatory thickening of the epidermis. Ingrowths into the thickness of the granulation layer no longer display their ordinary vertical anisomorphism. When, as a result of changes in the conditions of growth, they become distributed on the surface they again become more differentiated, and regain their vertical anisomorphism.

The left, unoperated leg shows only slight vacuolizing dystrophy of the epithelium.

Vascular changes due to section or stimulation of the sciatic nerve have been described in earlier papers [4]. Chronic stimulation of the nerve led to changes first of all in the outer, and in our experiments in the inner, part of the walls of the vessels. These changes consisted in appearance of large vacuoles below the nuclei of the endothelial cells of small arteries, which displace the nucleus towards the lumen of the artery (Fig. 2), and in hyperplasia of the tunica media of the vessel. The endothelial cells exhibited mitotic division, and gave rise to intravasal proliferation in small veins (Fig. 3). Proliferation of this sort has recently been reported in the endothelium of the lymphatic sacs of frogs [3], in the jugular veins of rabbits [7], and in the femoral arteries of dogs [5]. Apart from this, thrombosis and obliteration of skin blood vessels was noted. Capillaries developed in the granulation tissue of healing ulcers, together with relatively large thin-walled vessels made up of endothelium only. Both types of vessel developed from endothelial elements. Throughout all these changes the endothelial cells of the blood vessels remained distinct from connective tissue cells; in no case was their conversion into the latter, or their formation from the latter, observed.

Nerve trunks of all sizes, and nerve fibers exhibited the picture typical of Wallerian degeneration.

Unless the ulcerations were complicated by infection, leading to gangrene, we were able, even in the latest stages observed (over 6 months), to find healing of ulcers which had previously formed on the back of the paw. We found ingrowths of epithelium, resembling hair rudiments, under the flattened epidermis covering the site of the ulcer.

It is usually thought that development of ulcers following section of a nerve is due either to loss of tactile perception or to interference with regulation of local metabolic processes. It seems that the latter explanation is the more probable one; the effect may, of course, be reinforced by the effects of mechanical trauma suffered because of loss of sensation, as well as by infection of the ulcers forming.

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