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*Experimental Morphology*

# The Participation of Hemopoietic Bone-Marrow Derived Stem Cells in the Regulation of Damaged Cornea Stroma. Morphological Part

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**ABSTRACT. It is shown in the paper that regeneration of damaged cornea occurs due to hemopoietic stem cells of bone-marrow.**

**The above issue was studied by means of morphological, autoradiographic and immunofluorescence methods. By morphological method the formation of new structural elements – stretches – was found. These stretches grow from calcium vessel lying on the inner side of the limb. Stretches, which spread from eye's limb up to damaged infiltrate can be formed by means of migration of fibroblast-like cell, taking part in the regeneration of cornea's stroma. Epithelization of the damaged cornea occurs in 7 days, and regeneration of cornea of the stroma – in 30-40 days after operation. The results were obtained by histological methods.**

**Using autoradiographic method, it is shown that regeneration of the stroma of damaged cornea occurs not on account of the local stromal cells of the cornea itself, but on account of the cells settling into the cornea from outside. Thus, if we bear in mind that the cells getting into the eye are already labeled while in the blood vessels of the limb these labeled cells are single, we can assume that the source of the cells is outside of the limb. Hence we suppose that labeled fibroblasts take their origin in the bone-marrow. Non-direct autoradiographic data, obtained by us, can prove the bone-marrow origin of those cells.**

**The application of the immunofluorescence method to the xenogenic mouse radiation chimeras allowed us directly define the source of the cells taking part in the recovery of the damaged cornea. Namely, the greater part of fibroblast-like cells of infiltrate of the cornea of the xenogenic (rat-mouse) radiation chimeras were characterized by specific fluoroscence when treated with antiserum of the cells of rats' bone-marrow, pointing to the origin of these cells from transplanted cells of donor's bone-marrow, i.e. from hemopoietic stem cells.**


***Kew words**: stroma, limb, fibroblast-like cells, stretches, regeneration, xenogenic (rat-mouse) radiation chimeras.*

In the earlier investigations it was established that after damaging the cornea of rats the biggest number of mitoses was observed in two new layers of basal cells. The cells leaving the basal layer were directed vertically to the surface of the whole area of the cornea. The cellular flow from conjunctive to the cornea in limbal area was not observed. After the loss of contact by the cells with the basement membrane in a few days the division of the latter into layers without mitosis was recorded. The appearance of centrally localized cells is explained

only by migration of basal cells [1]. It was also shown that at perforated cornea the process of wound healing both in humans and in animals occurs due to epithelial cells and fibroblasts, formed by the transformation of the existing keratocytes [2]. Some authors assumed that fibroblasts in the centre of the wound of traumatic cornea appeared mainly due to transformation and distribution of the existing stromal cells [3-5]. The other part of investigators considered that most part of fibroblasts in infiltrate of stroma of traumatic cornea is formed due to transformation and division of monocytes penetrated into the wound from limb, and the lesser part generates from stromal cornea cells [6-8].

Regeneration of descemetos endothelium and descemetos membrane in the majoruty of vertebrates (rats, guineapigs, rabbits, primates) and humans occurs due to migration and mitotic division of endothelial cornea cells lying near to the edge of the wound [9].

According to a literary analysis of world investigations, most of the studies are devoted to the regeneration of the epithelium of damaged cornea and little is done concerning the stroma-cornea [10-13].

Most of the investigators consider that regeneration of the epithelium of the damaged cornea in men and in experimental animals occurs due to stem cells existing in basal the epithelium of cornea-scleral limb. Filling with new cells is explained by heterogeneity of the basement membrane in limbic zone [14-16] or by unique location of basic cell double domain of the limbo-corneal epithelium [17]. Some authors assrt that stem cells are located only in the basal layer of the outer vascularized limbal ring. It was counted and defined that limbo-corneal epithelium in mice is supported by 100 limbal stem cells only.

Scientists from Lausanne [18] established that everyday wear of cornea is compensated on account of the stem cells of cornea epithelium, while at serious cornea trauma stem cells are attracted from the limb of the eye sclera.

**Material and Methods**. The subjects of the investigation were white pubertal mice. Damage of the eye cornea in experimental animals was done by means of central, perforate wounding with sterile preparation needle.

In different periods after damage the lens was extracted, and the cornea was cut along the limb.

**Histologic methods**. For morphological investigations the operated corneas were fixed in 10% neutral formalin in 3, 6, 18 hours and further in a day up to 10 days after operation. 30- and 40-day morphologic preparations were also prepared. After histologic treatment of material the cuts were stained with hematoxylin-eosine according to Erlich by 3-colour Mallory method.

**Results and discussion**. The results of morphologic investigations showed that after full-layer perforated wounding of the eye cornea in white mice the exudative period of inflammation starts which is superseded by proliferate phase as a result of which infiltrate is formed.

In 3 hours after operation a wound with vertically cut edges across all the layers is observed in the central part of the cornea. At the same time new structural elements-stretches are formed. Usually they do not exist in the norm. These stretches grow from calcium vessel (the basis of blood supply of the inner parts of the eye) lying in the inner side of the limb. The stretches spread from limb to cellular infiltrate and only with in a day after operation they connect to the edges of the wound surface. Stretch consists of elongated cells situated parallel to each other. Among these cells there is a space, filled with blood elements. The stretches of the cells form a kind of capillary, through which blood cells from limbal vessels penetrate into infiltrate. In the same period between vertically cut edges of the upper part of the wound accumulation of structureless substance, sometimes with infiltrated cells, is observed.

On the surface of this structureless substance the epithelium cells are located in one incorrect layer, on the

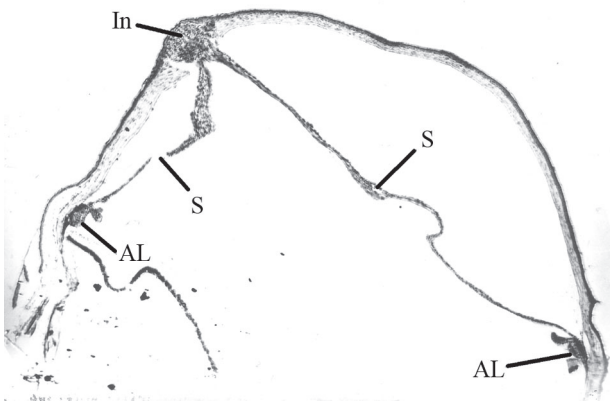
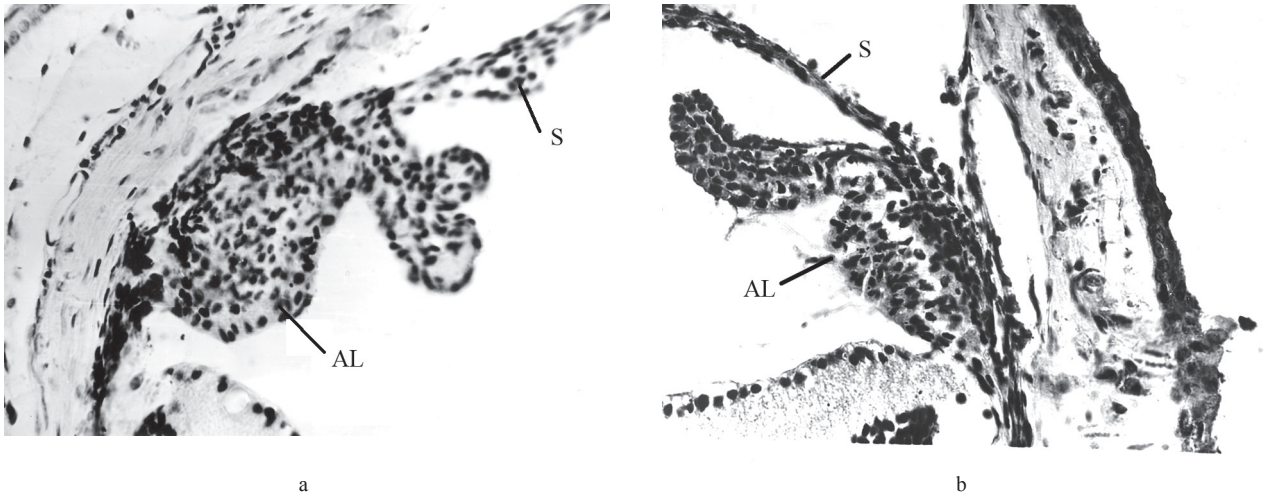



Fig. 1. Cross-section of adult mice eye cornea. Five days after operation. Cellular infiltrate and the stretches formed in traumatic cornea, Infiltrate (In) takes up the most part of wound; the stretches (S) go to the infiltrate from the limbal area (AL). Hematex and eosine staining, magnification - X25.




a b

Fig. 2. Cross-section of adult mice eye cornea. Five days after operation. Limb area (AL) where stretches (S) directly pass to the limbal tissues, a. left side; b. right side; Hemat. eosine staining, magnification: a, b- X400.

other edge of the wound the epithelial layer is strongly thickened and grows into structureless substance. In the lower edge of the structureless substance accumulation of elongated cells is marked.

On the 2-3 day after operation, unlike on the 1st day, better formed stretches are obtained going from the limb up to the lower wound edge. These cellular stretches grow from the fold of ciliary body as 1-2 layer spindle-shaped cells.

In 7 days the wound is completely epithelized. The epithelium has a typical structure of flat cornea epithelium. The seam between the regenerates epithelized surface and epithelium of intact cornea is not observed. Cornea stroma is not yet completely regenerated. There is only a thin layer of the main substance. The remaining part of the damaged cavity is filled by cellular infiltrate, from which stretches spread, directed into different sides. First they go from the limb across the surface of the outer front edge of the cut of the cornea. In the limb area stretches directly go into limb tissue. The stretch itself consists of elongated cells.

In 6-7 days after wounding the cornea sroma is partially regenerated. It contains spindle-shaped cells. From the lower edge of stroma as in the earlier stages after trauma, the cellular stretches direct to the limb; wound cavity is filled with cellular infiltrates.

On the 9th -10th day of wounded cornea the biggest part of the stroma is regenerated. The histologic structure of a newly formed stroma, which consists of parallel collagen tissues and fibroblasts, differs little from the intact tissue. Fibroblasts have not clearly expressed spindle-shaped form and in some places are oriented not quite parallel. Besides, regenerated stroma does not fill the whole wound.

Descemet membrane and descemet endothelium are not regenerated. At the lower edge of the regenerated stroma the stretches of the cells are still present. They connect the infiltrate with the limb area. At this stage these cellular stretches do not have such dense structure as in the earlier stage after operation. Later, on the 30th and 40th day after operation the stretches become empty, infiltrate is friable and stroma is completely regenerated (Figs. 1, 2).

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