MORPHOLOGY AND PATHOMORPHOLOGY

Quantitative Characteristics of the Recycling of Corneal Receptors

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A recycling of receptors in the intact rabbit cornea is revealed using the Golgi method. The process includes spontaneous autotomy and degeneration of certain terminal branches accompanied by the regeneration of others. In order to synchronize the regeneration, an ultraviolet burn of the cornea is performed. By means of automatic image analysis it is shown that 7 days after the burn new receptors appear that have an area of the receptor field equal to 56% of the control, an area of receptor membrane equal to 59% of the control, and a density of innervation equal to 150% of the control. Toward the 15th day hyperregeneration is observed in both ultraviolet-exposed and shielded regions of the cornea. It is speculated that regeneration is stimulated by tissue proteases.

Key Words: *tissue receptors; regeneration; cornea; consequences of ultraviolet radiation; automatic image analysis*

The cornea is an organ with multiple peculiarities. It has no blood or lymphatic vessels, glandulocytes, or myocytes, and consists 80% of water. The cornea has no specific target cells for innervation; however, it possesses a rich plexus nervosus and multiple receptor fields [2]. At the same time, it apparently does not represent a special sensory organ. Therefore, it is to be assumed that the nervous apparatus of the cornea, including its receptor part, executes an efferent trophic function aimed at maintaining the peculiar crystalloid structure and extremely high regenerative capacity of this organ. In view of this, it seems useful to analyze the regenerative capacity of the receptor apparatus following injury and regeneration of the corneal structure.

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MATERIALS AND METHODS

The experiments were carried out on adult chinchilla rabbits. Synchronized receptor regeneration was induced by a dosed corneal burn with ultraviolet (UV) radiation. A DRT-100 (PRK-7) mercury-quartz lamp served as the radiation source. An eye was exposed to radiation for 15 sec at a distance of 50 cm. Radiation power was 258.613 W/m^2, which exceeds the sanitary standards 4-5 times. The nervous apparatus of intact and experimental animals was studied using the method of silver nitrate impregnation after Golgi. Changes in the corneal nervous structures were followed up 4, 7, and 15 days after UV radiation. A study of the dynamics of quantitative parameters that characterize the structural organization of the cornea was conducted using a television-based automatic image analyzer (IRIS-V). The receptor field area (RFA) and the area of outlined field directly occupied by receptor elements (OFA) were measured. The re-

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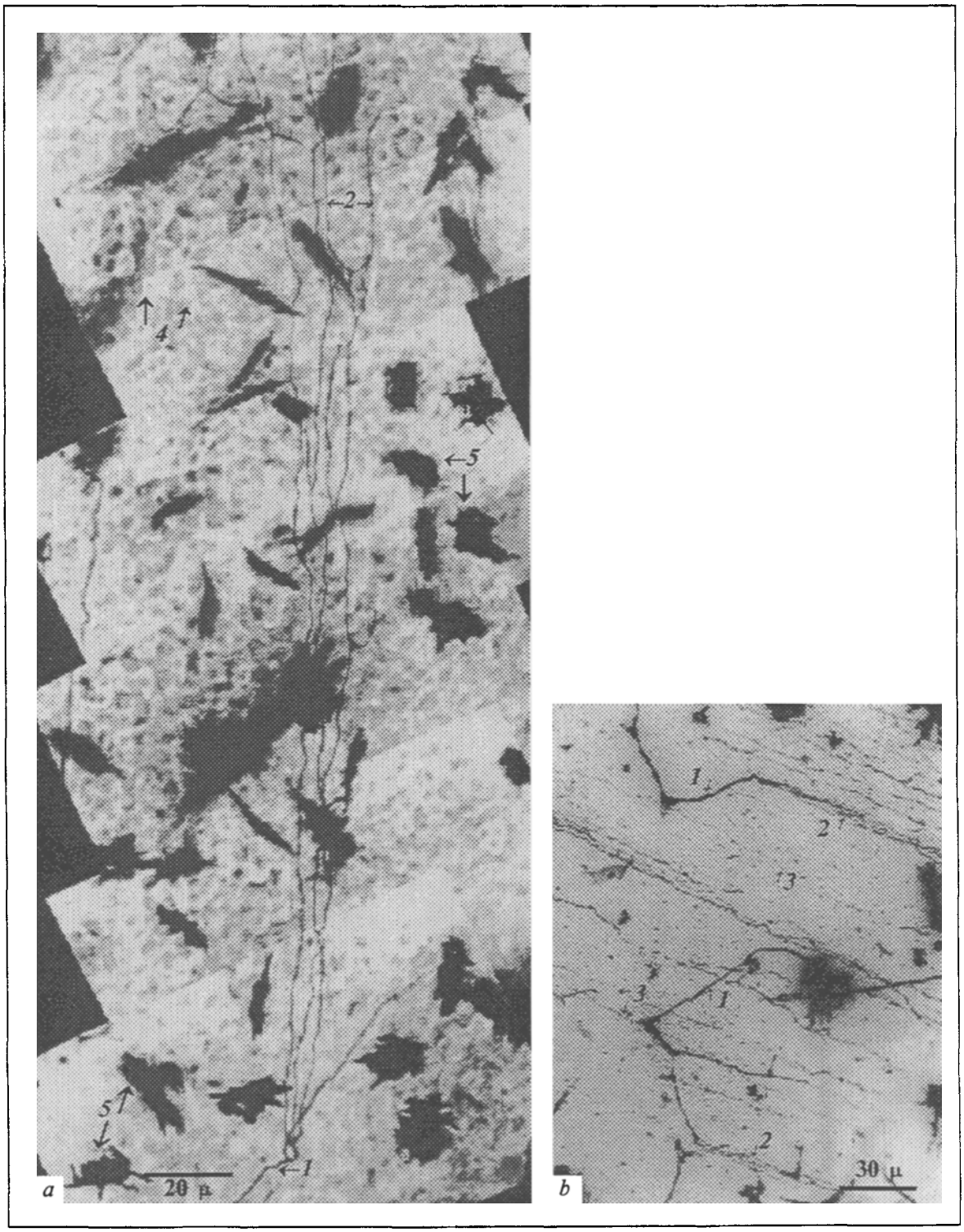

Fig. 1. Sensory nerve endings in the cornea of intact rabbit. *a*) solitary bushlike receptor; *b*) receptor fields with fragments of degenerating endings in the normal cornea (receptor recycling); *1*) initial fiber; *2*) terminal fibers; *3*) degenerating endings; *4*) epitheliocyte outlines in the cornea; *5*) keratocytes. Impregnation after Golgi.

ceptor membrane area (RMA) and density of innervation,i.e., the ratio of RMA to RFA, were calculated in the control and after UV radiation. In the course of RMA estimation, the terminal receptor fibers were considered as cylinders and the area of their surface, i.e., the membrane area, was calculated according to the formula: $RMA=2\pi RH$, where R is the radius and H is the length of the

cylinder ($H=OFA/2R$); thus, $RMA=2\pi R\times OFA/2R=\pi\times OFA$. A total of 58 receptors from 32 animals were analyzed.

RESULTS

The terminal nervous apparatus of the corneal epithelium consists of multiple free branches of the terminal brush-shaped neurites (Fig. 1, *a*). The receptors are characterized by the presence of a single, compact zone of branching. Secondary radicles are relatively rare.

Analysis of the normal corneal nervous apparatus reveals an abundance of receptor fields (Fig. 1, *b*); moreover, these receptors express a number of specific structural features. Thus, in the intact animals a considerable number of terminals show marked reactive alterations (Fig. 2, *a*) that are expressed as multiple deformations. Isolated and degenerative terminals also occur (Fig. 1, *b*) in the vicinity of neurites bearing growth cones. These data suggest that in the normal cornea two opposite processes occur simultaneously, namely, spontaneous destruction and regeneration of receptors, i.e., active receptor recycling. Apparently, such a physiological regeneration of corneal receptors cannot be explained exclusively by the shedding of keratinized epithelium [5]. Phenomena of receptor degeneration and recycling are also observed in the internal enteric plexus [5] and in the corneal stroma [2]. We propose that synchronization and prolongation of receptor recycling can be achieved by a one-shot amputation of all receptors using UV radiation of the cornea.

Morphometric analysis of receptors in the control (intact) cornea revealed significant variations in their parameters. The mean index of RFA was 731.5±75.0 μ^2 and the mean area of the sensory membrane of a single receptor was 318.1±35.8 μ^2. The high heterogeneity of the mentioned indexes may reflect a heterochronous pattern of receptor self-amputation in the course of recycling. The mean innervation density (RMA/RFA) is considerably more uniform (nearly 0.43). Its individual variations are three times smaller than those of RFA and OFA. In light of these results the form of the receptor and the absolute value of RFA may be less essential for receptor functioning than the innervation density (the size of sensory membrane area per unit of innervated substrate area). The innervation density is known to be an indirect indicator of the nervous system-mediated trophic influence that governs the intensity of tissue regeneration [4].

Four days after UV burn all receptors and their initial fibers undergo degeneration. However, new, regenerating receptors appear as early as on the 7th day. RFA attains 407.0±45.2 μ^2 (55.6% of the control), and the area of the profiles of receptor elements attains 59.9±4.9 μ^2 (59.1% of the control). This is explained by the fact that at this time the number of receptor branches is smaller than in the control. At the same time, the density of innervation reaches an even slightly higher level (0.46). Thus, in this period the degree of receptor maturity is about 56-59% and the density of innervation (possibly of a trophic nature) is higher as compared to the control. The high density of innervation may be due to the requirement for intensified trophic support of the regenerating corneal tissue.

The degree of variability of the above-mentioned parameters slightly decreases after the burn. All receptors appear to start recycling from zero. However, by the 7th day variability within the receptor structures reaches a considerable level. Ten days after burn multiple cones of receptor branch growth can be seen (Fig. 2, *b*). On the 15th day the development of receptor neurites markedly exceeds that in the control (Fig. 2, *c*). RFA rises to 1304.3±104.9 μ^2 (178% of the control), and OFA to 286.0±20.8 μ^2 (282% of the control). Total RMA reaches 898.0±65.3 μ^2 (282% of the control). Hyperregeneration of receptors is also manifested in a considerable increase of terminus branching and in the appearance of numerous growth cones. As a result, the receptor develops from a thin brushlike structure into a broadly branched bush. An objective indicator of hyperregeneration is the increase of the density of innervation by more than 1.5 times (0.69).

Hyperregeneration of the nervous apparatus during this period is also clearly expressed in the stromal plexus, where short processes with growth cones develop. It is worth noting that the expansion of neurites proceeds in parts of the cornea unexposed to UV (shielded). Moreover, capillary regeneration and intervention into the cornea from the limbus take place. Thus, one can assume that the phenomenon of receptor hyperregeneration is mediated not by the direct effect of UV radiation, but rather via a certain humoral mediator that possesses properties of both neurotrophic factor and vascular growth factor.

Such properties are inherent in the proteolytic enzymes that can dramatically enhance neurite regeneration and branching [1], and activate the proliferation of endotheliocytes. It is known that after burn the activity of proteolytic enzymes reaches a high level in the cornea as a whole. This suggests that tissue proteases serve as mediators of

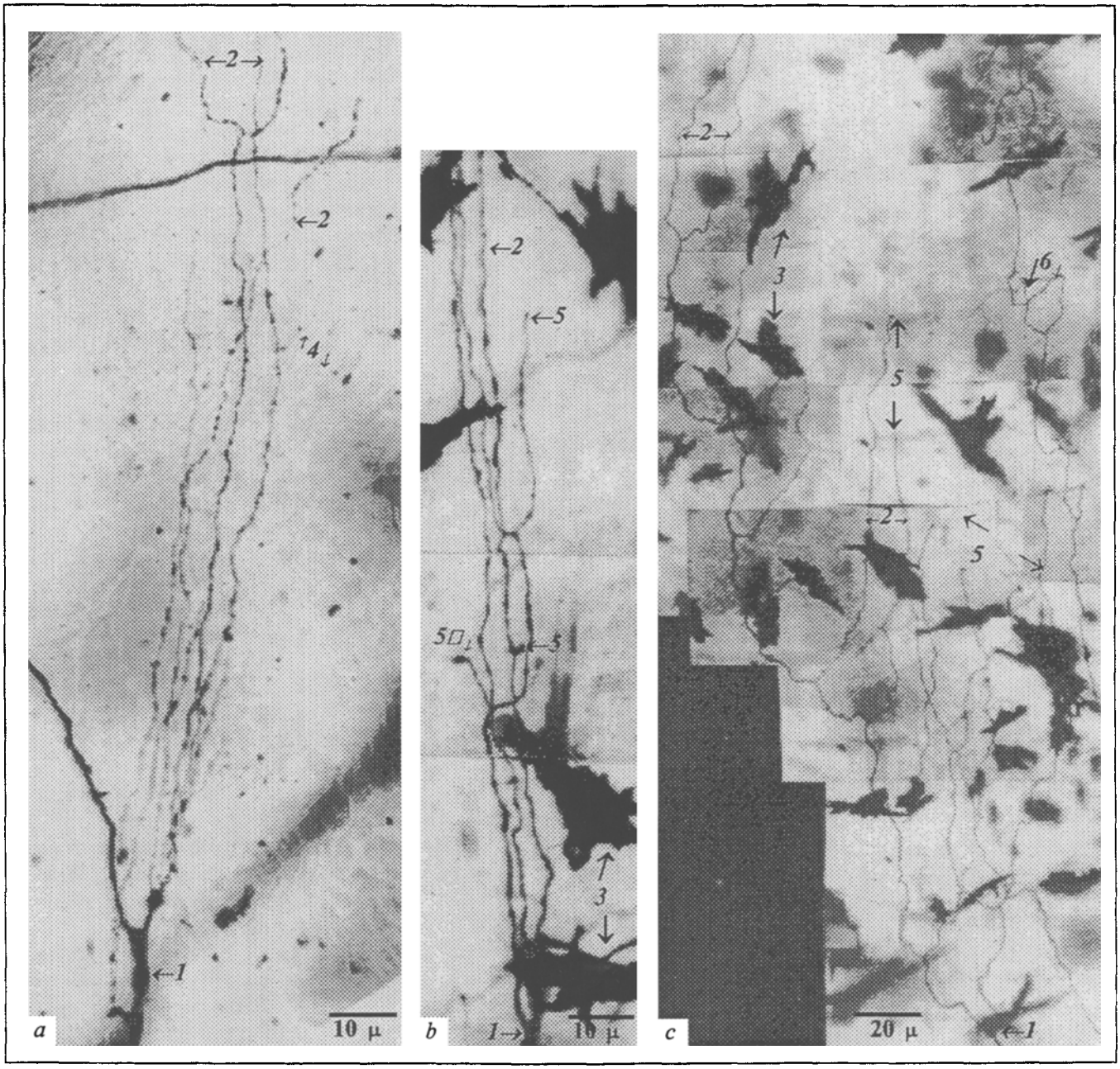

Fig. 2. Dynamics of receptor morphology at different stages of regeneration after UV–induced corneal burn. *a*) receptor with varicose alterations of endings in the control; *b*) multiple growth cones at an early stage of receptor regeneration (10th day after burn); c) receptor hyperregeneration 15 days after burn. *1*) initial fiber; *2*) terminal receptor fibers; *3*) keratocytes; *4*) epitheliocyte outlines in the cornea; *5*) growth cones; *6*) circular structures of regenerating fibers.

nervous regeneration and hyperregeneration in the cornea. It is likely that in the intact cornea pronounced regeneratory activity is also supported by the trophic effect of activated lysosomal hydrolases that are released from amputated and degenerating receptor fragments during their recycling.

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