ON CERTAIN CHANGES IN THE DEGREE OF CORNEAL TISSUE DIFFERENTIATION UNDER EXPERIMENTAL CONDITIONS

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 56, No. 7,
pp. 99-103, July, 1963
Original article submitted January 2, 1962

Problems in regeneration, blastomatous growth, and to some degree – vascular pathology, are all linked to resolving the question pertaining to the conditions that affect a change in the degree of tissue differentiation. A very convenient subject for studying these questions is the avascular corneal membrane, which has been used many times in investigations, since it makes it possible for the most graphic demonstrations of the effect of a nerve apparatus on its tissue [15, 19, 20, 21].

In a number of works [8, 11-15, 18] it was shown that injury to the trigeminal nerve causes neuroparalytic keratitis, which arises both with the correction of external injuries [13] and with the removal of reflex influences [3, 14], but the pathogenesis of the changes that arise in the cornea in this case has not been fully clear up until now.

The indicated authors studied the dystrophic process in the cornea in connection with an effect on the trigeminal nerve.

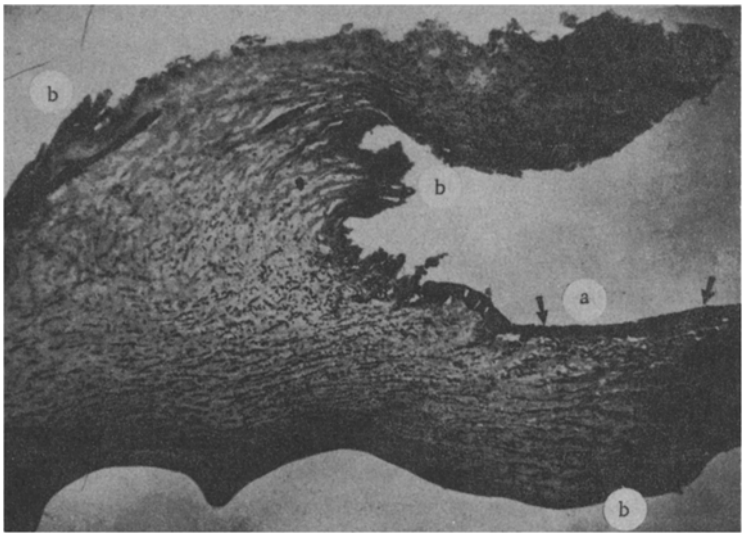

Fig. 1. Tissue from the central area of the cornea after annular electrocoagulation (experiment No. 36). a) Site of the burn; b) "polyp"; ab) normal thickness of the cornea. Period of observation – 27 days. Impregnation with silver, according to the method of Campos. Obj. 3.5 ×, ocul. 10 ×.

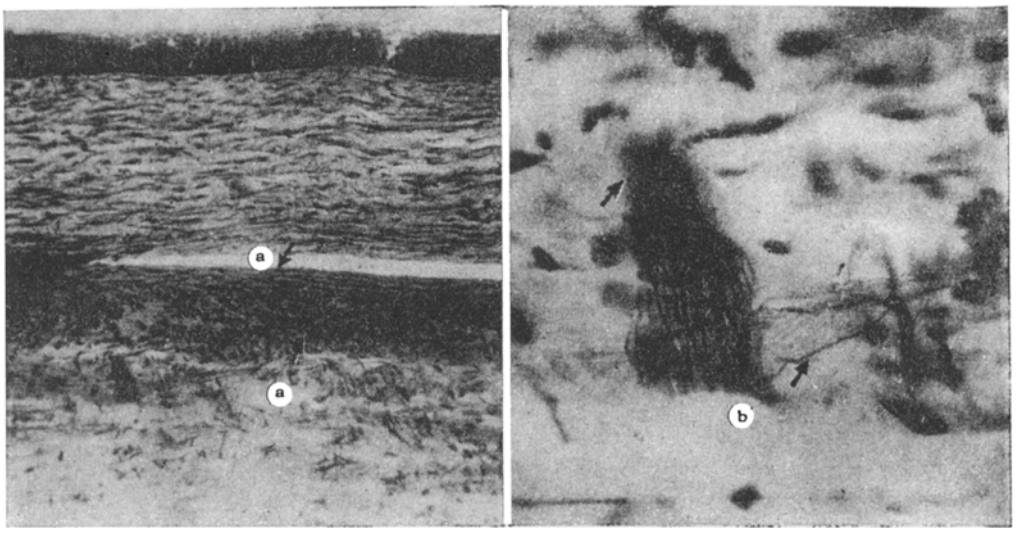

Fig. 2. Corneal tissue (experiment No. 41). Period of observation – 34 days. a) Transplant in a pocket between the superficial layers of the corneal stroma. Obj. 8 ×, ocul. 7 ×. b) Nerve fibers growing into the transplant. Obj. 40 ×, ocul. 10 ×. Impregnation by the method of Campos.

Considering the peculiar structure of the neural apparatus in the cornea, we attempted, in previous investigations [4, 5, 6] and in the present work, to study the change in the degree of its tissue differentiation during an experimental action directly within the confines of the corneal membrane itself. Without suggesting that this is an exhaustive explanation, we present the preliminary data of these investigations.

EXPERIMENTAL METHOD

Using the cornea of adult rabbits (176 experiments), we investigated a number of techniques, influencing the change in its tissue differentiation.

The material was subjected to histological treatment, with subsequent impregnation of the nerves by the method of Bielshowsky-Gross, modified by Lavrent'ev, by the method of Campos, and with different histological stains.

In 50 corneas, following local anaesthesia with a 0.5% solution of dicaine, we performed an annular electrocoagulation (optical electrode, 0.5 mm in diameter) at the central portion of the corneal membrane of the rabbit (diameter of the ring – from 5 to 8 mm). The mild, reactive irritiation of the eye normally disappeared in the next 1-2 days.

EXPERIMENTAL RESULTS

Turbidity of the central area of the cornea was common to these experiments. The periphery of corneal membrane surrounding the circular burn retained its transparency, and the eye remained undisturbed in the course of the entire period of observation (up to 4 months).

The ultimate changes in the central area depended on the depth of the circular cauterization. A superficial burn caused an intermediate heightening of the central area. A deep burn, up to half the thickness of the corneal layers, led to marked proliferation of the tissue in the central area.

With repeat trauma, trephination along the ring of the burn or secondary cauterization, the proliferative processes were even more marked. The tissue of the central area of the cornea in this case, losing its functional properties (elasticity, transparency), became cloudy, plastic, and acquired the ability to grow rapidly, giving rise to its form of "polyp" (Fig. 1).

In the histological picture during the first few days, the elements of edema predominated, with a simultaneous increase in the cellular composition of the corneal stroma. Characteristically, there was a decrease in the differentiation

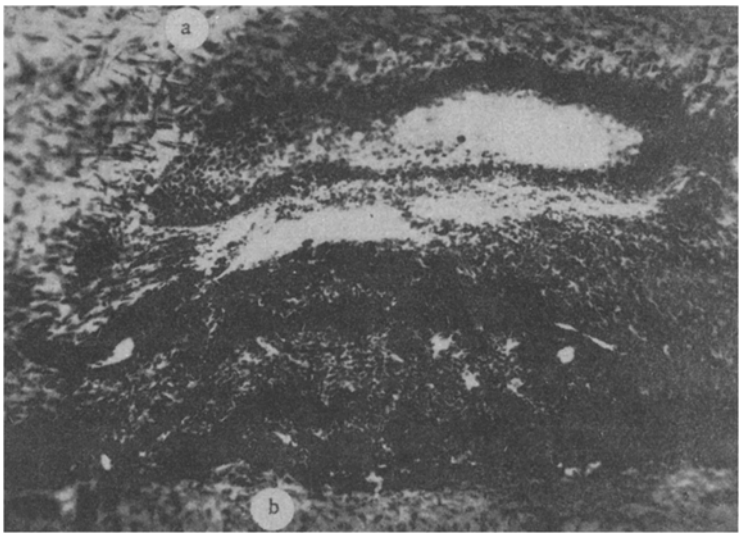

Fig. 3. Transplant in the pocket between deep layers of the corneal stroma (experiment No. 31). Period of observation – 33 days. a) Superficial layers of the stroma; b) deep layers of the stroma; ab) transplant (tissue culture in vivo). Impregnation by the method of Campos. Obj. 8 ×, ocul 10 ×.

of the tissue in this area of the cornea, and the nuclei of the cells changed from their elongated form to an oval, or even round, shape. This process of unique "rejuvenation" of the corneal tissue occurs more intensely from the 7th-12th day, and is accompanied by rapid increase in the tissue volume – manifest proliferation.

On investigation the nerve elements, we noted their injury at the site of the annular corneal burn. In the cornea, the basic nerve plexuses run in the upper half of its layers; thus, with cauterization, they are subjected to maximum destruction. New nerve trunklets are not observed in the young, proliferating tissue; we only encountered rare, old, degeneratively altered, nerve branches. Ultimately, as the defect at the site of the annular burn was filled in, nerve fibers appeared, growing in from the peripheral portion of the transparent cornea to the newly formed tissue at the site of the burn erosion. Gradually, the central zone of proliferation began to decrease, as though melting into the periphery. This became marked after filling and tissue maturation at the site of erosion from the circular burn. The process normally took $1\frac{1}{2}$-2 months, depending on the depth and width of the burn line. Thus, concentrically, beginning from the periphery, there occurs a clearing and differentiation of the corneal tissues, the last step being an alteration of the very center, or – with an irregular burn – of the area nearest to the deepest and widest burn site.

At this time, we were usually able to trace the penetration of the newly arising nerve branches from the peripheral, uninjured cornea. With secondary circular injuries, the process was repeated more intensely, and with marked proliferative growth. The corneal tissue seemed to reorganize more quickly after the repeated injury, "releasing the brake" on its growth.

In the other portion of the experiments, we used the method of transplanting a corneal membrane, with incomplete differentiation of the tissue (cornea of a newborn rabbit), between layers of transparent cornea in an adult rabbit.

A round transplant, 4 mm in diameter, from the cornea of a newborn rabbit, was placed in a pocket prepared (after an oblique slit in the superficial layers of the cornea) by cleaving the layers of the stroma with a blunt instrument. The operation was carried out under local anaesthesia. The pocket was prepared either nearer the surface layers, or between the deep layers, of the corneal stroma. The results of the experiments differed. The transplant placed between the superficial layers differentiated and cleared more rapidly, blending with the surrounding stromal layers. In a number of the preparations in this series of experiments, we could see nerve trunklets entering into the transplant, or large nerve trunks coming close to the tissue of the transplant (Fig. 2).

This is not surprising, since, according to the data in the literature [1, 2, 7, 9, 10, 16, 17] and our own observations, the neutral apparatus is richest in the superficial half of the corneal layers, and considerably poorer in the deeper layers, where only individual nerves are found.

The transplant in the pocket between the deep corneal layers developed differently. Enlarging markedly, it filled the entire volume of the pocket and expanded the adjoining boundary layers of the stroma. The tissue structure of the transplant also changed; we observed a decrease in the degree of differentiation of its elements. In a number of cases, the transplant changed into a culture of the tissues from which it was taken (Fig. 3). The neighboring stromal tissue, surrounding the transplant, also underwent a shift toward a decrease in the degree of differentiation, and became markedly thickened; this thickening, and the expanding transplant, were noticeable even macroscopically, in the form of a cloudy swelling, up to 6 mm in diameter, against a setting of surrounding, transparent cornea.

On histological investigation, we were unable to trace neutral elements either into the tissue of the transplant, or into the stromal layers below. At the same time, there were a number of nerve branches in the superficial layers of the cornea, but they branched off into the bordering stromal layers, also not penetrating into the pocket.

Our observations serve as a basis for postulating a definite "autonomism" in the tissues of the cornea, which is only manifested when they lose their connection with the nervous system, the latter inhibiting the growth and division of the cells. There occurs a decrease in the degree of differentiation of the tissue cells, an increase in the rate of growth, and subsequently, even conversion to an unusual in vivo tissue culture.

The experiments showed that an increase in the degree of tissue differentiation in the cornea develops in parallel with the ingrowth of nerve endings; it may be postulated that the degree of differentiation and the functional adequacy of the tissue are maintained by the neutral apparatus.

SUMMARY

Circular burn of the corneal surface reduces the differentiation and induces proliferative growth in the central area in the form of a "polyp". Transplants from the cornea with incomplete development, mature rapidly, after being placed between the superficial layers of the corneal stroma. Nerve branches growing into the transplant are visible in the preparations impregnated according to Campos. In the same transplants placed between the deep corneal layers, where the nervous apparatus is poor developed, the degree of differentiation is decreased and follows the type of the tissue culture in vivo. It is suggested that the degree of the tissue differentiation and its functional adequacy are maintained by the nervous apparatus, whereas denervation provokes tissue "autonomism" , i.e., reduction of differentiation and acceleration of tissue growth.

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