EXPERIMENTAL BIOLOGY

A STUDY OF THE PROLIFERATIVE PROPERTIES OF CORNEAL TISSUES DURING WOUND HEALING

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The cornea of the eye has long been considered by scientists to be a valuable subject for the study of many biological problems [12, 13, 21, 23].

The theory that regenerating epithelium is unilaminar before it becomes stratified [17, 19, 24] has been critically analyzed by N. A. Shevchenko [16] and can now be considered baseless. However, many things regarding the structure of regenerating epithelium and especially of its growth edge, are not yet sufficient clear.

Epithelial proliferation is regarded by some authors as an active process [12, 16, 24, 27] and by others as a passive process [1]. Some authors do not believe that the whole epithelial sheet is displaced; these authors believe that regeneration is caused by the movement of the basal cells alone to the top of the sheet [22]. There is a theory that epithelial regeneration is caused by the disintegration of the sheet into cellular elements which have a reproductive or defense-support function [2, 3, 18].

According to some authors, the actual substance of the cornea is regenerated both from the local connective tissue cells and from the cells emigrating from the eye tissues surrounding the cornea [4, 6, 8]. Other authors [19, 20] give more significance to either the connective tissue cells of the limbus, which migrate to the focus of regeneration, or the local corneal cells [9, 10, 11]. There was another theory expressed by certain authors [14, 25, 27] some time ago that the connective tissue cells of the cornea formed from the epithelium.

Although many authors [4, 7, 9, 16] admit that epithelium is more active than connective tissue, it has also been shown that the tissues must proliferate simultaneously [17] and that there must be a definite correlation between the regeneration of the epithelial tissue and that of the connective tissue [8]. The theory that differentiation of the corneal epithelium is associated with the development of Bowman's membrane [5] is not sound, since a structurally expressed Bowman's membrane does not exist in some animals.

EXPERIMENTAL METHOD

The experiments were done on 62 rabbits. First, the cornea was anesthetized with a 2% solution of cocaine hydrochloride and irrigated with a weak solution of boric acid; then a wound was inflicted with a trephine as much in the center of the cornea as possible. The epithelium and the connective tissue were injured; the corneal defects were made in the form of holes 2 mm in diameter. The cornea was examined 1, 2, 4, 6, 12 and 18 hours as well as 1, 6, 8, 14 and 30 days after the operation. The material was fixed in Zenker's fluid. The paraffin and celloidin sections were mostly stained with Heidenhain's iron hematoxylin with an after-stain of Mallory's mixture or Bemer's hematoxylin with picroindigocarmine.

EXPERIMENTAL RESULTS

The first reaction of the tissues to the wound consisted of disturbance in the metabolic processes and the development of a reactive inflammation, more or less expressed. In the epithelium this reaction was characterized

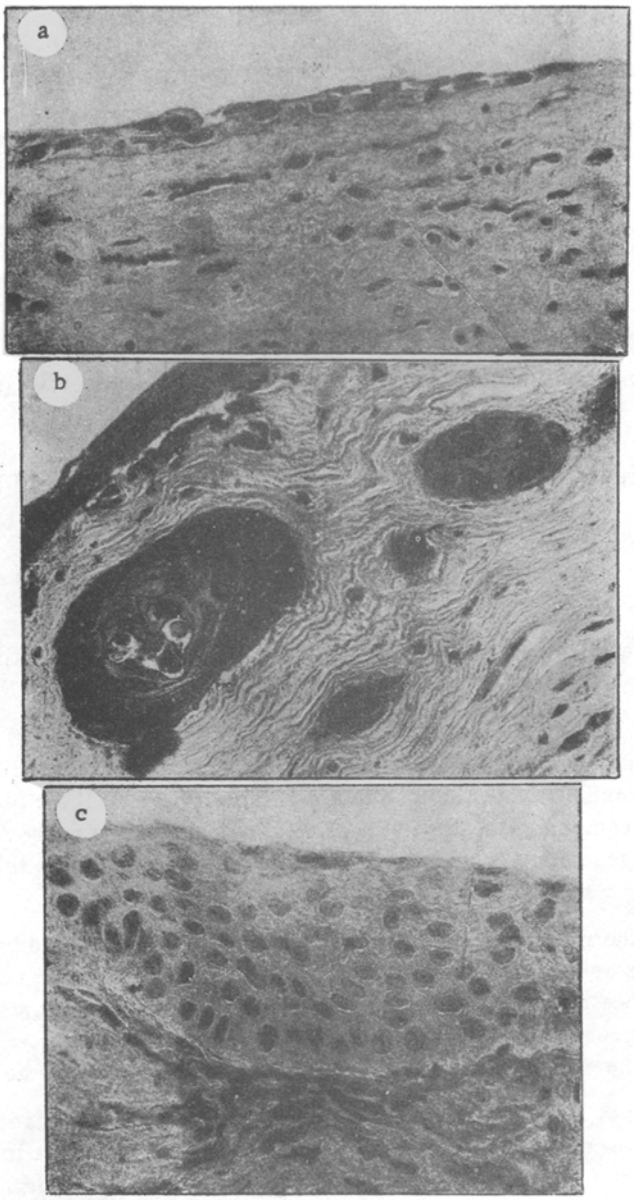

Fig. 1. The process of proliferation of the epithelium of the cornea in a rabbit. a) Single-layer wedge of regenerating epithelium on destroyed connective tissue 4 hours after operation. Fixative: Zenker's fluid. Stain: Carazzi's hematoxylin and eosin. Magnification: apochromatic obj: 2 mm, comp., ocular 5 x. b) Ingrowth of epithelium into connective tissue in the form of a band 4 days after operation. Fixative: Zenker's fluid. Stain: iron hematoxylin. Magnification: apochromatic obj: 2.2 mm, comp., ocular 5 x. c) Accumulation of fibroblasts under newly formed epithelium. 6 days after operation. Fixative: Zenker's fluid. Stain: Bemer's hematoxylin and picro-indigocarmine. Magnification: apochromatic obj; 2.2 mm, comp., ocular 5 x.

morphologically by a disturbance in the stratification. Some of the less differentiated cellular elements became activated and lost any sign of functional differentiation. Other elements, which had already started the differentiation process and were incapable of activation, quickly degenerated. The theory that this process consists of two phases – first, the formation of a unilaminar epithelial regenerate and then its transformation into stratified epithelium [17, 19, 24] – was not confirmed in the experiments conducted. But the actual wedge of epithelial growth often consisted of only a few layers, its edge sometimes even being unilaminar (see figure).

During the proliferation of the epithelial sheet the tissue connections were weakened and the intercelluiar relationships changed. The edge of the epithelial sheet became wedge-shaped (see figure). At the beginning of proliferation the shape of the edge depended both on the character of the wound and on the type of reorganization, a phenomenon connected with the degree of activation.

Later in the proliferation process the degree of stratification depended on the intensity of the epithelial growth. The faster the growth of the epithelium, the thinner its edge. At first epithelization was due primarily to activated cells from the surrounding epithelium causing the edge of the latter to become much thinner. Changes in the shape of the cellular elements also caused the epithelium to become thinner in the marginal zone.

Most of the cells divided by mitosis; division occurred first in the unchanged epithelium, with mitoses being found in the activated zone of the epithelium only 10-12 hrs later some distance from the edge of the regenerate. The migration of cambiogenetic* cells into the marginal zone described by some authors [2, 3, 17, 18] does not occur in actual fact.

Although the connective tissue of the cornea was less reactive (in the sense of its proliferative properties), considerable changes were observed in it from the moment the wound was inflicted. Edema and leukocyte infiltration developed, disturbing the laminar distribution of the collagenous fibers.

The activated epithelium grew over and smoothed out this uneven connective tissue.

Under conditions of wound regeneration it is comparatively difficult to observe the relationship between the differentiation of newly formed epithelium and the condition of the underlying tissue. The epithelial regenerate began differentiation very early. The new epithelium began to differentiate at the 24-hr stage, before the wound was completely epithelized and before connective tissue proliferation had started; a basal layer of tall cells and a second layer of polymorphic and flat cells could be discerned in it. These data contradict the theory [5] that epithelial differentiation begins after the appearance of Bowman's membrane. We found that the epithelium could lie either on the nonproliferating connective tissue or on the newly formed connective tissue and have in either case a definite laminar polarity. A definite relationship with the underlying tissue is evidently established during epithelial differentiation, the disturbance of which can cause the epithelium to grow into the underlying tissue (see figure, b).

The main substance of the cornea was regenerated from the connective tissue cells of the cornea proper. However, the connective tissue cells of the limbus could also participate in the regeneration process, especially when the corneal defect was near the limbus. Therefore, it would seem that in the cornea there were both differentiated corneal connective tissue cells (fibrocytes) and less differentiated cells [5, 17], which reacted to the wound in different ways. The fibrocytes of the injured area disintegrated. Activated fibroblasts migrated from the uninjured area under the connective tissue surface into the regenerating connective tissue. This was attended by the constant dehydration of the regenerating tissue and by a decrease in the number of leukocytes. The number of fibroblasts increased and accumulated under the regenerated epithelium (see figure, c). The fibroblasts divided both mitotically and amitotically; division was first observed 48 hrs after the operation.

The old collagenous fibers disintegrated in the sections where the fibroblasts had accumulated, and new intercellular fibrous structures formed. The disintegration of the old collagenous fibers, the special leukocytes [17] participating, began with the inflammatory process and subsequently proceeded in the presence of lymphocytes and histiocytes.

The zone of the disintegrating corneal connective tissue was gradually filled with newly formed tissue. For a long time this regenerate consisted of intercellular matter and cells of the fibroblast type. The laminar structure was not expressed; the newly formed fibrous structures, in the form of fibrils and bundles, were unorientated and remains of the old fibers were found among them.

* As in original.

Therefore, the regenerated portion of the cornea had not yet acquired the structure typical of an uninjured cornea at the latest stages of regeneration which we examined.

SUMMARY

The process of healing of the injured cornea was investigated in rabbits. This process was followed up for the period ranging from 1 hr to 30 days after infliction of the injury. Biphasic course of regeneration of the epithelium and migration of cells from the epithelial layer to the border zone was not revealed. It was demonstrated that the structure of the epithelial wedge depended on the conditions of the experiment. Connective tissue possesses a lower reactivity than epithelium and it regenerates with its own cells. When the wound is inflicted near the limbus the elements of the latter may take part in its regeneration.

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ANALYSIS OF THE MECHANISM OF DEGRADATIONAL MITOGENETIC RADIATION

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Degradational mitogenetic radiation arises in living objects during moderate cooling (2-5°C), light anesthesia, centrifugation (2500-3000 rpm), the application of a weak (0.02-0.05 ma at 4-6 v) constant or alternating current, or spontaneously, as, for example, in nerve and muscle systems [1, 2, 3].

An analysis of this phenomenon led A. G. Gurvich to the conclusion that in the substance of living objects there are molecular constellations at a high energy level for the maintenance of which statistically uninterrupted influx of energy is required, and that consequently these molecular constellations are not in equilibrium.

According to Gurvich [4], these unbalanced molecular constellations consist of general levels of energy along which absorbed energy migrates and possibly is summated by the constellations. During disturbance of the constellations this energy is partially emitted in the form of degradational radiation, with a spectrum which is determined by the unbalanced state of the molecular constellations. According to A. G. Gurvich's physiological theory of protoplasm [3], the unbalanced molecular constellations constitute the fundamental reactive apparatus of living systems.

We thus see that the importance of studying degradational mitogenetic radiation lies in the fact that first by its appearance or absence during the application of degrading factors to a living object it is possible to judge the intrinsic energy level of that object; in the second place the spectrum of this radiation may give some idea of the state of the general reactive apparatus of living systems – on the state of the unbalanced molecular constellations and on the mitogenetic condition of the nerve and muscle systems.

The above indicates the considerable general biological importance of the study of degradational mitogenetic radiation. However, in the mechanism of its production there is much that still remains unexplained.

The investigation to be described was devoted to the study of the duration of degradational radiation and of the ability of the unbalanced molecular constellations to accumulate energy and to summate small quanta of it. As the subject of the investigation we used the degradational radiation of the liver of the mouse and of the layer of an onion, caused by cooling, and the method of demonstrating it was by the use of biological detectors.