EXPERIMENTAL HISTOLOGICAL STUDY OF THE EPITHELIA OF THE ANAL AREA IN RATS

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Epithelia of different tissue types are characterized by specific morphological and functional properties which are determined by their different origins and development during phylo- and ontogenesis. A regular change of epithelia is seen in many locations on the body. A distinction should be made [8, 9] between border areas formed by epithelia of the same type of tissue and epithelia belonging to different tissue types. In the first case a gradual changeover is possible from one epithelium to the other, in the second case such mutual transformations or changeovers are impossible and a seam is formed.

When fistulas are formed when skin flaps and some other operations of plastic surgery are made the formation of new border areas between different epithelia and the establishment of new interrelations is observed [6, 7]. These important problems still remain inadequately studied from a histological point of view, although they are of great interest in surgical pathology.

In the present work we studied the changes of the skin and intestinal epithelia in the anal area during regeneration.

EXPERIMENTAL METHOD

The subjects of the investigations were white rats. Under ether anesthesia, a contiguous area (3 X 5 mm) of both epithelia which line the dorsal wall of the anal canal was injured in 109 animals by excision or cautery with a heated metallic plate or thermocautery.

The materials for the investigation was taken at times after injury – from 2 hours to 10 months – and fixed with Zenker's fluid, 15% formalin and Buoin's fixing fluid, and then covered with celloidin. Serial sections 6-7μ in thickness were stained with Heidenhain's iron hematoxylin (sometimes with supplementary staining with mucicarmine), hematoxylin-eosin, hematoxylin with supplementary staining with mucicarmine and aurantia azure II-eosin and azan solution.

In addition to the sections, flat preparations were made by macerating fresh material or by the colloidal film method (of Kochetoc), as well as by the mechanical removal of the outer layers of the intestinal wall. The preparations were fixed with formalin and stained with hematoxylin and mucicarmine.

EXPERIMENTAL RESULTS

The wound in the vicinity of the seam was edged partly with epidermal and partly with intestinal epithelium in all cases. Usually the submucosa was its base; in some cases the injury included the muscular layer.

Following the injury, reactive changes (occurring on top of and in connection with the developing inflammatory process in the connective tissue below) occurred which led to complete closure of the defect and the reestablishment of relations close to normal [2].

The special reconstruction, or "mobilization" [10], of each of the epithelial layers around the wound, their active movement over the surface of the wound, and mitotic cell division have significance in the reparative regeneration of the epithelia.

It is important to observe that, in spite of some difference in the clinical course, histologically there was no major difference between the epithelia, whether the defect was produced by excision or cautery. However, excision allowed clearer study of the progress of epithelial regeneration, especially in the early stages after the injury.

Reactive changes of the epidermal epithelium began only a few hours after the injury. The form and orientation of the cells changed, which led to some thinning of the layer around, widening of the intercellular spaces and lengthening of the cytoplasmic bridges.

At the same time, mitoses occurred in the epithelium at a distance from the edge of the wound. They were found in the epidermis, as well as in the epithelium of the hair follicles and sebaceous glands.

The edge of the regenerated epidermis became wedge-shaped (in section) 4-15 hours after the injury. This stratified sheet later moved like a border over the surface of the wound along the fibrinous exudate and injured connective tissue (Fig. 1). The regenerated tissue was formed from the previous epithelium and is its direct continuation. The vertical anisomorphism of the regenerated area was not expressed as clearly as usual

Fig. 1. Epithelial regeneration in the seam area of tegumentary and intestinal epithelia in the anal area. Two days after excision. Zenker's fluid, hematoxylin-mucicarmine-aurantia. Drawing somewhat schematic. x 400.

at first: not infrequently the stratum corneum and stratum granulosum were absent, the cellular cytoplasm of the thickened growing zone was basophilic, a fact which was, apparently, due to the presence of ribonucleic acid [5]. There was no basement membrane under the regenerated tissue soon after the injury.

Approximately 2 days later, simultaneously with the formation of granular tissue, distinct signs of secondary differentiation appeared in the regenerated epidermis. The latter gradually spread away from the original epithelium toward the free edge of the moving sheet. A basement membrane appeared: the cells of the regenerated epithelium acquired a more regular shape and orientation, the superficial elements became cornified, although the stratum granulosum may be lacking. Mitoses appeared in the regenerated tissue itself, although they were previously absent in it; they occurred in the basal layer and the first layer or two above it. The edge of the regenerated tissue remained less differentiated during the whole process of the epithelialization of the wound, until the new raphe was formed. Later secondary differentiation of the epidermal regenerate was quickly completed.

In contrast to the tegumentary type of epithelium, reconstruction of the simple intestinal epithelium took place in such a way that, along with a decrease in the height of the cells and their spread along the surface, the topography of the mucosa also changed and there was a redistribution of cellular material. Similar reconstruction was observed in the epithelium of the small intestine [4]. Already a few hours after injury some of the crypts nearest the defect took on a slanting position, their orifices were turned toward the wound (Fig. 1). At the end of the first 24 hours, the crypts shortened as though opening, and their epithelium "leaked" over the surface of the wound.

The regenerated epithelium, a continuation of the former intestinal epithelium, is one layer constantly and throughout its extent (Fig. 1). The accumulations of cells which sometimes appeared in it, especially at

its free edge, did not at all resemble the striated epidermal sheet with its typical vertical anisomorphism. Among the flattened regenerated cells there were cells which contained mucous, but which were not goblet-shaped. The basement membrane was absent under the regenerated tissue in the early stages.

After 1-1½ days the zone of increased mitotic activity, at first located at a distance from the defect, gradually moved toward it and in 2-3 days it extended to the edge of the wound.

Fig. 2. Completion of the regeneration of the tegumentary and intestinal epithelia in the anal area, 12 days after excision. Zenker's fluid, hematoxylin-mucicarmine-aurantia. Drawing ×400.

The regenerated tissue was located at first on the fibrinous exudate or on the former connective tissue which had been changed by inflammation, later on the newly formed granulation tissue.

Fig. 3. Region of the new raphe 12 days after excision. Flat preparation. Formalin, hematoxylin-mucicarmine. Drawing × 80.

As the edge of the simple epithelium moved over the surface of the wound, it differentiated gradually, spreading from the original epithelium to the free edge of the sheet covering the wound. The cells became taller, the intestinal border appeared, the amount of mucous inclusions increased, superficial pits appeared which gradually changed into crypts. A basement membrane appeared. Secondary differentiation of the regenerated intestinal epithelium was quickly completed after a new raphe of the epithelia was formed, which usually occurred by the 8-10th day after the injury (Fig. 2).

Usually the area over which the striated epithelium spread was greater than that of the intestinal epidermis. As a result, the majority of the defect was covered by the tegumentary epithelium, while the new raphe usually occurred where the intestinal epithelium had been located, as can be seen best in the flat preparations (Fig. 3).

During the regenerative process, both epithelia in the anal region were observed to grow down into the underlying inflamed connective tissue. The growth of the tegumentary and intestinal epithelia differed greatly, in accord with available data [2].

Later developments, which occurred in the epithelia as well as in the connective tissues, led to the gradual, very slow reestablishment of the histological relations peculiar to the normal anal raphe area [2].

On the basis of the above, as well as of bibliographic data [3, 8] the metaplasia of the intestinal epithelium into the tegumentary and of the tegumentary into the intestinal, instead of their natural raphe, can be denied.

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