IN VIVO CULTURE OF THE EPITHELIUM OF THE EPIGLOTTIS

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The epiglottis stands high among the various divisions of the larynx in respect of the incidence of carcinoma [7, 10]. For this reason the study of the biological properties of the epithelium of the epiglottis, its reactivity and its plasticity, is of considerable interest. Several workers have studied the histogenesis of this organ [3, 6, 9, 13-16], although the problem of the origin of the epithelial surface of the epiglottis remains unsolved at the present time. According to Shimkevich's metarhisis theory [12], the epithelium of the foregut in the embryo and its derivatives are ectodermal in nature. However, the results of various experimental studies, using Lazarenko's implantation method, of the organs of the foregut of embryos [1, 2, 5, 11] have not confirmed this theory. The general properties of these organs, as revealed after implantation, indicate that they arise from a prechordal anlage. The epiglottis has not been investigated in this context.

EXPERIMENTAL METHOD

We studied the epithelium of the epiglottis by the method of tissue culture in vivo described by Lazarenko [4, 8]. Three series of experiments were performed. The recipients in all the experiments were adult rabbits aged 5-6 months. The donors in the experiments of series 1 were rabbit embryos before birth, series 2—rabbits aged 3-4 weeks, and series 3—adult rabbits aged 5-6 months. The implants were extirpated at intervals of between 6 h and 50 days after the beginning of the experiment, fixed in Zenker's formol fluid, and embedded in paraffin wax or celloidin. Sections were cut to a thickness of 4-6 μ and stained with Mayer's hematoxylin, by Böhmer's method, with eosin and with picro-indigocarmine. In addition, PAS-positive substances not destroyed by amylase were investigated by McManus's method, as components of tissues indicating the degree of their functional activity and differentiation. Altogether 75 implants were studied.

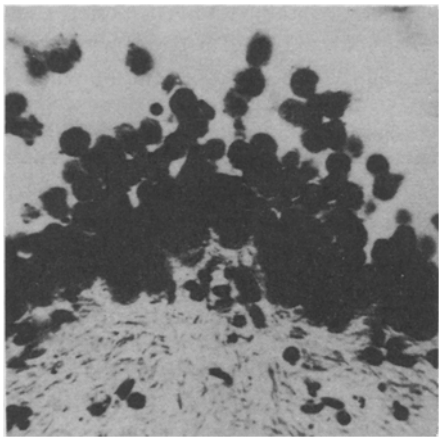

Fig. 1. Culture of epithelium of the epiglottis on 1st day of implantation. Photomicrograph. Mayer's hematoxylin—eosin. Ocular 2, objective 90.

EXPERIMENTAL RESULTS

Several general principles emerged from the study of the tissue changes in all 3 series of experiments. During the first hours of implantation the tissues of the donor's epiglottis showed depression of activity. Subsequently they became permeated with inflammatory exudate, their activity was stimulated, and they began to proliferate. In the stratified epithelium on the surface of the organ these changes affected only the cells of the 2-3 basal layers (Fig. 1). The more superficial cells matured more quickly and became detached from the surface. Separation of cells into those undergoing activation and those undergoing degeneration was also found in the glands of the epiglottis. Meanwhile, between the pieces of celloidin, a new and richly vascularized connective tissue began to infiltrate, supplying the growing epithelium with its necessary nutriment.

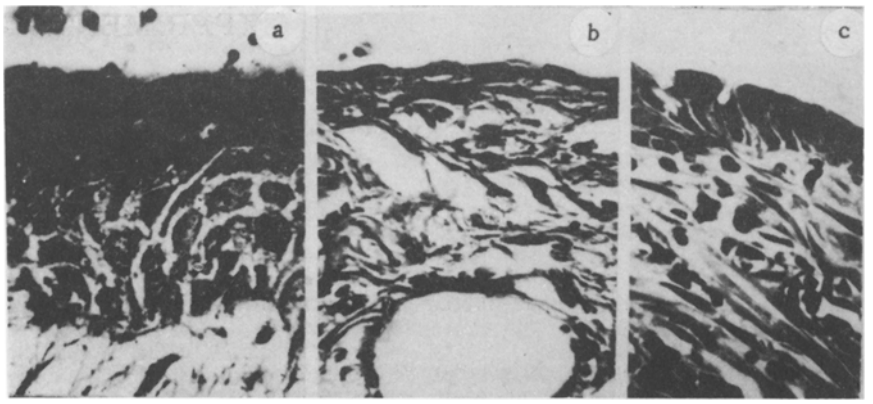

Fig. 2. Culture of epithelium of the epiglottis. a) On 10th day of implantation; b) on 5th day; c) on 6th day. Photomicrograph. McManus (a); Mayer's hematoxylin–eosin (b and c). Ocular 2, objective 90.

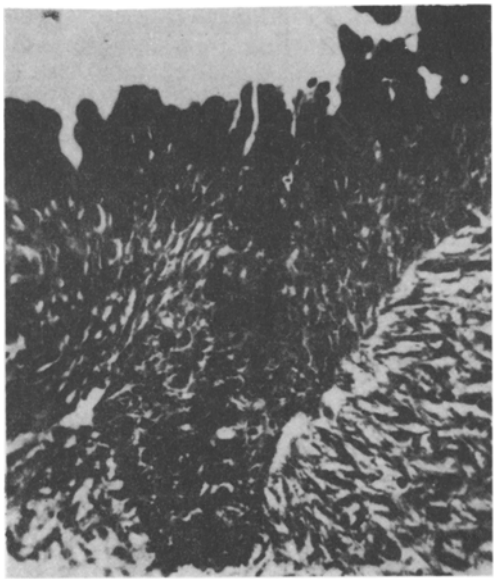

Fig. 3. Culture of epithelium of epiglottis on 8th day of implantation. Photomicrograph. McManus. Ocular 7, objective 40.

On the second day of implantation the activated epithelium of the superficial layers and glands began to proliferate. It spread first over the surface of its own piece of tissue, sometimes completely surrounding it so that the piece died; the epithelium then changed into the newly formed connective tissue of the intercelloidin layers and grew as sheets and bands. The bands were directed towards the source of stimulation—the celloidin or the necrotic masses. Having reached them, they spread out into multilayered or more rarely into single-layered sheets. The newly formed sheets grew around the pieces of celloidin and necrotic masses and isolated them from the recipient's body tissues. This was a manifestation of the phylogenetically most primitive, protective properties of the epithelium.

On the 4th-5th day, organ-forming processes began to take place in the implanted tissues. With continuing inflammation, the epithelium of the newly formed sheets began to invade the underlying connective tissue in the form of downward growing bands. The sheets and bands of epithelium as they began to develop were completely undifferentiated in character. Their cells possessed high mitotic activity. They contained no PAS-positive substances, and their apical and basal portions possessed equal growth potential, and the sheets tended to fuse where they came in contact.

On the 6th-8th day after the beginning of the experiment, side by side with substance of the inflammatory process, differentiation of connective tissue and newly formed epithelial structures began to take place in the implant, shedding light on the properties of the epithelium and on its potential capacity.

In the process of differentiation of the epithelial sheets, different forms of epithelium were produced. Where they came in contact with the solid celloidin, sheets of stratified epithelium were formed (Fig. 2, a). Initially undifferentiated, they acquired vertical anisomorphism and accumulated PAS-positive substance in their superficial layers, i. e., they differentiated along the lines of the protective type. In areas protected from the traumatic action of the celloidin, sheets of single-layered, cubical, squamous (Fig. 2, b) or multilayered (Fig. 2, c) epithelium appeared.

The stratified and multilayered epithelial sheets showed a tendency towards glandular differentiation. Goblet-shaped cells, producing a mucoid secretion, began to appear (Fig. 3). Sometimes these were numerous and were

grouped into structures resembling alveoli, actually lying within the sheet. They stained an intense red color by McManus's method, and this did not disappear after treatment with amylase.

Differentiation of the epithelial bands invading the spaces between the celloidin developed along the lines of formation of both protective, stratified structures and glandular structures. In the first case the central cells of the bands acquired polarity. As a result of a mutual redistribution of the cells in the center of the band a lumen appeared. The band was converted into a tube, lined with a stratified squamous, nonkeratinizing epithelium, the superficial layers of which accumulated PAS-positive substances. In the second case the bands ramified and were converted into structures resembling the tubulo-alveolar glands or the epiglottis, with their characteristic double-layered arrangement of the myoepithelial and secretory cells in their terminal portions. On the 6th-8th day of implantation, some secretory cells of the terminal portions began to function. Often atypical differentiation of glands took place in the bands. The central cells of the band began to produce a mucoid secretion. Under pressure of the accumulating secretion they died, forming a cavity so that the band was converted into a cystic structure, filled with mucoid contents.

The transformations described above were demonstrated more fully when the epithelium of the epiglottis from 3-4 week rabbit embryos was cultivated. The epithelium of the adult rabbit yielded a less polymorphic structure: stratified sheets were predominant, fewer glands were present, and these showed greater atypism.

On the 12th-13th days of implantation the newly formed epithelial structures began to undergo a process of regression. This was due to several causes, the chief of which was cicatrization of the connective tissue of the implant, so that it could no longer maintain the normal trophic conditions of the functioning epithelium.

Epithelium of rabbit epiglottis was cultivated in vivo by Lazarenko's method, using adult rabbits as recipients and rabbit embryos (3-4 weeks) and adult rabbits (5-6 months) as donors. The epithelium of the epiglottis revealed high reactivity and plasticity, as shown by active growth followed by the formation of stratified, multi-layered and single-layered sheets and glandular structures. These properties of the epithelium of the epiglottis diminished with age. Because of the character of growth of the epithelium of the epiglottis when cultivated in vivo, and its high plasticity, similar to that of other organs derived from the foregut, it may be classed as an epithelium derived from a prechordal anlage with a wide range of biological potentialities.

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