CHANGES IN PHARYNGEAL EPITHELIUM WHEN CULTIVATED IN VIVO

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Stratified squamous and compound ciliary epithelium of the rabbit's pharynx were cultivated in vivo by Lazarenko's method. Epithelial structures grew in the implanted tissues: sheets of tissue consisting of single or multiple layers, and buried bands. Organ structures appeared during the period of differentiation of the cultures: stratified layers with a protective zone, compound ciliary epithelium with goblet cells and secretory tubules. A wide range of plasticity is a biological feature distinguishing the pharyngeal epithelium, which evidently develops from the prechordal plate.

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Information concerning the morphology of the pharyngeal mucous membrane is incomplete and contradictory [2, 4, 5, 9-12]. The genetic nature of its epithelial lining, like that of the epithelium of the whole of the foregut, remains unexplained [1, 6-8].

In the present investigation we attempted to study the biological properties of the epithelium of various parts of the pharynx under experimental conditions and to determine its genetic nature. We used F. M. Lazarenko's method [3] of in vivo tissue and organ culture for this purpose, for it enables the potential capabilities of the epithelium to be ascertained.

EXPERIMENTAL METHOD

Because of the morphological and physiological features distinguishing the various parts of the pharynx, homoimplantation of the mucuous membrane of the laryngopharynx and nasopharynx were carried out separately. The donors were rabbits aged 1 month and the recipients adult animal. Altogether 56 experiments were performed. The implants were extirpated at intervals of from 1 to 40 days, fixed in Carnoy's fluid, and embedded in calloidin–paraffin. Sections were cut to a thickness of 5-7 μ and stained with Mayer's hematoxylin and eosin, Bohmer's hematoxylin and picro-indigocarmine. Glycogen and neutral mycopolysaccarides were detected by the Hotchkiss–Shabadash method, acid mucopolysaccharides by Hale's method, and nucleic acids by Brachet's method. Appropriate controls were used for differentiation of the detected compounds.

EXPERIMENTAL RESULTS

During the first hours of the experiment changes took place in the region of the implants exactly as usually occurs in tissue cultures in vivo.

During the first day of the experiment highly differentiated cells of the upper protective zone of the layer in implants of the mucous membrane of the laryngopharynx matured rapidly in the stratified epithelium and became detached (Fig. 1, 1). Only the cells of the lower 2-4 layers of the stratum germinativum, containing considerable amounts of nucleic acid, changed into a proliferative state. The activated cells at first moved along the connective tissue of the fragment itself, and then along the newly formed connective tissue of the recipient toward the source of stimulation (the celloidin and necrotic masses of implant), forming layers at the border with them. The stratified squamous epithelium of the laryngopharynx proliferated in the form of stratified or single-layered sheets containing flat or cubical cells (Fig. 1, 2). The growing sheets consisted initially of undifferentiated epithelial cells. They synthesized large quantities of nucleic acids and were always located on undifferentiated connective tissues containing a large amount of

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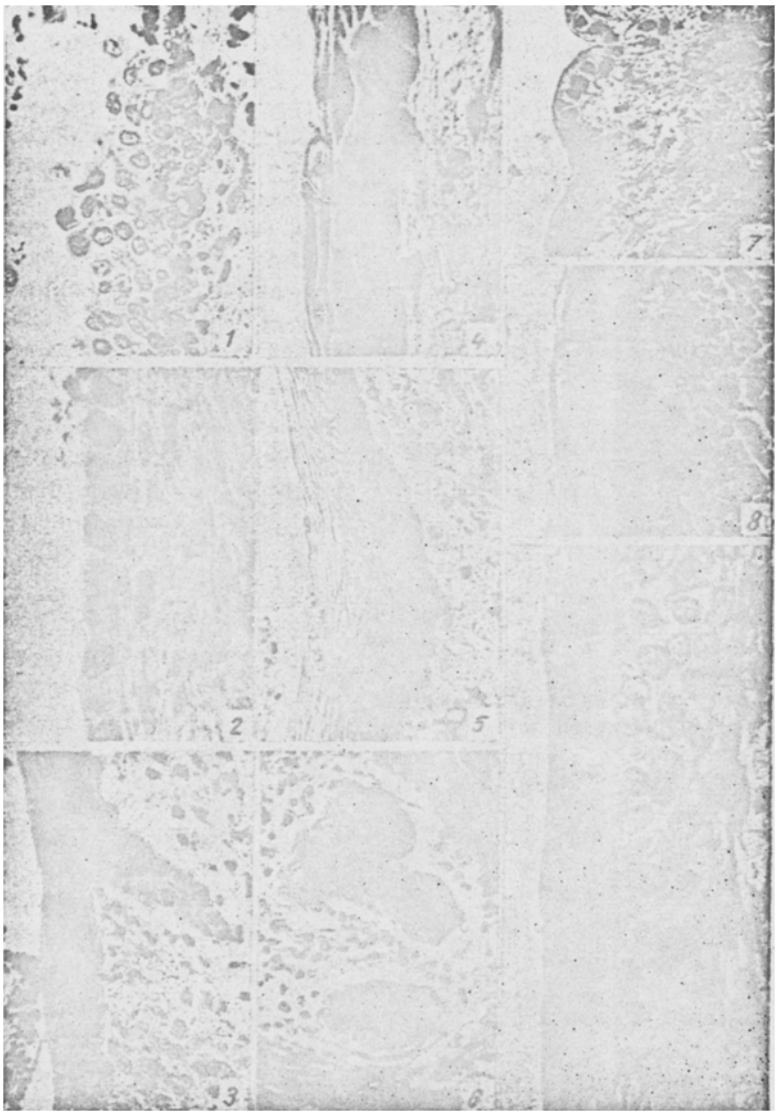

Fig. 1. Cultures of laryngopharyngeal (1-5) and nasopharyngeal (6-9) epithelium. 1) 2 days. Fixation by Carnoy's method, staining with hematoxylin-eosin, objective 90, ocular 7; 2) 5 days. Fixation and staining the same, objective 90, ocular 7; 3) 8 days. Fixation and staining the same, objective 40, ocular 7; 4) 5 days. Fixation the same, staining by Brachet's method, objective 90, ocular 7; 5) 6 days. Fixation and staining the same, objective 40, ocular 7; 6) 14 days. PAS reaction, treatment with amylase, fixation and magnification the same; 7) 3 days. Staining with hematoxylin–picro-indigocarmine, fixation and magnification the same. 8) 12 days. PAS reaction, treatment with amylase, fixation and magnification the same; 9) 15 days, fixation and staining the same, objective 90, ocular 7.

hyaluronic acid. Later the single-layered epithelium changed into stratified. In the period of acute inflammation, bands grew out from the layers in the implant into the subadjacent young connective tissue (Fig. 1, 3), their structure being similar to that of the original sheet. The buried bands consisted of young and, as is clear from Fig. 1, 3, mitotically active cells. These bands terminated freely in the connective tissue, or, having reached the source of stimulation, opened out to form a layer separating the connective tissue from the foreign bodies and necrotic masses.

On the 6th-8th day of the experiment, inflammation in the implant subsided. Meanwhile proliferation of the tissues diminished and differentiation of the newly grown structures began. This took place simultaneously in the epithelium and connective tissue of the layers between the celloidin. In these layers the quantity of amorphous substance rich in hyaluronic acid diminished while the number of fibrous structures increased. In the stratified sheets the numbers of layers of cells increased. They acquired the property of synthesizing glycogen and mucopolysaccharides. A definite order was observed in the synthesis of these substrates. In a young sheet glycogen and RNA appeared first (Fig. 1, 4). With the appearance of mucopolysaccharides, concentrated at the periphery of the cells of the apical layers, the glycogen content diminished and RNA was concentrated in the cells of the lower layers of the sheet (Fig. 1, 5). The stratified epithelium became separated into two zones: an upper (protective) and lower (germinative) zone. The buried bands freely ending in the connective tissue differentiated into glandular epithelium. Tubes with secretory cells functioning as mucous glands formed from them. These glandular structures were found in tissue cultures of the mucous membrane of both the laryngopharynx and nasopharynx (Fig. 1, 6).

During cultivation of the mucous membrane of the nasopharynx, its compound ciliary epithelium differentiated during the first day of the experiment. The epithelium became activated and began to proliferate towards the end of the first day, forming sheets of both single and multiple layers (Fig. 1, 7).

The single-layered epithelium changed into multiple or stratified, subsequently indistinguishable from the same epithelium in implants of the laryngopharyngeal mucous membrane. On the 6th-8th day of the experiment, differentiation of the multi-layered epithelium was accompanied by the formation of cilia and of goblet cells containing large quantities of mucopolysaccharides in their cytoplasm (Fig. 1, 8). RNA was distributed uniformly in the cytoplasm of all cells of the sheet. The multi-layered epithelium in the early stages of implantation could also be converted into stratified, with subsequent differentiation. Sometimes biochemical substrates accumulating in different parts of the same stratified sheet led to differences in the character of differentiation. Areas of protective epithelium and of stratified secretory epithelium with goblet cells appeared (Fig. 1, 9).

The connective tissue of the implant became denser after 15 days. It did not provide trophic conditions for functioning epithelial structures, so that these died and were absorbed.

The compound ciliary epithelium of the nasopharynx and the stratified epithelium of the laryngopharynx thus exhibited considerable lability when cultivated in vivo. Stratified, single-layered and multiple-layered sheets with the ability to undergo conversion from one end to the other grew from them.

Compared with other parts of the organ, the epithelium of the nasopharynx possesses the greatest plastic properties. Besides stratified, single-layered, and multiple-layered structures, compound ciliary and stratified secretory epithelium grew from it.

The manifestation of high plasticity is a biological feature distinguishing the pharyngeal epithelium, providing for the complex functions of this organ at the crossroads of the digestive and respiratory systems, and also gives evidence of the special genetic nature of its epithelium, which evidently develops from the prechordal plate.

LITERATURE CITED

1. A. G. Knorre, and V. P. Mikhailov, Arkh. Anat., No. 1, 3 (1961).
2. Ts. F. Kossovskii, Histology of the Proximal Portion of the Digestive Canal, Dissertation, Warsaw (1880).
3. F. M. Lazarenko, Principles Governing Growth and Conversion of Tissues and Organs during Cultivation (Implantation) in Vivo [in Russian], Moscow (1959).
4. V. A. Malishevskaya, Pharyngeal Glands of Man and Some Animals, Candidate Dissertation [in Russian], Khar'kov (1952).
5. A. A. Ovsepyan, Comparative Histology of the Oropharynx of Cattle. Candidate Dissertation [in Russian], Erevan (1955).
6. P. G. Svetlov, Arkh. Anat., No. 4, 7 (1963).
7. N. G. Khlopin, General Biological and Experimental Basis of Histology [in Russian], Leningrad (1946).
8. V. M. Shimkevich, Izv. Akad. Nauk, Ser. 6, No. 12, 997 (1903).
9. M. Y. Ali, J. Anat. (London), 99, 657 (1965).
10. C. Chauveau, Le Pharynx, Paris (1901).

11. H. Luschva, Der Schlundkopf des Menschen, Tübingen (1868).
12. S. Schumacher, in: W. Möllendorf (editor), Handbuch der Mikroskopischen Anatomie des Menschen, Vol. 5, Part 1, Berlin (1927), p. 290.