## EXPERIMENTAL BIOLOGY

## DEGENERATIVE AND RESTORATIVE PROCESSES IN THE OLFACTORY ORGAN OF FISH AND AMPHIBIANS

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The olfactory sacs of animals (40 Crucian-carps and 40 axolotls) were damaged by means of thermocautery, or the olfactory bulbs of the brain were damaged with the aid of a knife. In the olfactory sacs immediately after the operation considerable destruction and exfoliation of the damaged section of the receptor layer of the olfactory lining was observed. Coagulation of the tissues in places spread to the underlying connective tissue, covered with blood clots and necrotic mass scab.

On the 4-6 and 8th day an intense restorative reaction on the part of the underlying connective tissue was found. On the damaged edges of the olfactory lining falling away of particular degenerative elements was observed. In the axolotl the lumen of the nearest Bowman's glands, as a rule, were distended. In these glands we observed intensive secretory processes with subsequent secretion of the controls on the surface lining. In the parts nearest the site of the burn the olfactory cells, as a rule, were subject to degeneration. Here their peripheral outgrowths underwent a peculiar foliated decomposition. The nucleo-plasmatic body was intensely stained. Finally the residues of the olfactory cells either underwent phagocytosis or were eliminated in the lumen of the olfactory sac.

Nevertheless part of the olfactory cells can later apparently restore their structure and function. The sections of multi-nuclear ciliated epithelium, next to the lesion separating the olfactory bulbs from each other and also the nearest supporting cells were greatly multiplied mitotically. In such sections the receptor layer of the olfactory lining maintained for a while a multi-nuclear structure and individual surface elements were ciliated. Only at the very edge of the lesion did the cells lose their proper orientation and were heaped on each other; towards the 10-15th day they started to move forward in the form of a peculiar wedge along the connective tissue, lifting thereby the necrotic masses of the scab. Occasionally such a layer of cells was situated on the substratum of the fibrin which, in a fine film, covered the lesion of the receptor layer. Thus, restoration of the intactness of the receptor layer of the olfactory lining was effected both by the active movement of the epithelial and supporting elements and by their proliferation. It is necessary to note the participation in this process in the axolotl of the terminal sections and chiefly of the secretory ducts of the Bowman glands. Occasionally the glands were transformed into cysts, overfilled with secretion and their walls, by rupturing themselves, might have served as the source for the newly grown integument, as was observed with skin damage upon regeneration of the multi-layer epithelium and its derivatives.

On the 20-25th day the damage had been fully eliminated. The new cover was an epithelio-like lining. It was built out of multi-nuclear, and occasionally a multi-layer, stratum. In 3-4 months the damaged parts of the olfactory sac in the investigated animals were covered with multi-nuclear ciliated epithelium. In the axolotl, individual glands partly preserved and apparently partly newly formed were observed, as is the case in healing of cutaneous wounds in mammals [1].

In all the stages studied in the Crucian-carp and in the axolotl restoration of the wrinkled "contour" on the damaged surface of the olfactory sac was not observed. Thus, the integument elements of the olfactory

lining of the fish and amphibians, in particular, the sections of the multi-nuclear ciliated epithelium and also the supporting cells possess the capacity, by interaction during regeneration with the underlying connective tissue, of actively replacing the damage.

It should be stressed that in the regenerated integument reformation of the olfactory cells was never seen either in the Crucian-carp or in the axolotl. The olfactory cells were maintained only in the undamaged parts of the olfactory sac. In the experiments of other authors [4, 5] the preserved parts of the olfactory lining have been looked upon apparently, as newly-formed ones.

One may say that in those cases where as a result of unsuccessful operation almost the entire lining of the olfactory sac was destroyed, the epidermis of the cutaneous integument, from that part of it which borders on the nostril, may participate in the healing of the damage.

The changes in the olfactory nerves with burning were marked by slowly developing Wallerian degeneration. A large number of nerve fibers for 10-15 days were subjected to decomposition. Some elements of the Schwann membrane showed high mitotic and amitotic multiplication.

The nuclei of the majority of gliar cells were elongated in length, often assuming rod-like shape. The vessels were considerably dilated and over-filled with formed blood elements. The leucocytes passed from the vessels to the nerve tissue, infiltrating it and phagocyting the decomposing nerve fibers.

Inflammatory processes, but less in intensity, were also found in the region of the olfactory bulbs of the brain. They expressed themselves in a dilation of the vessels and infiltration of the nerve tissue. On the 10-20th day, part of the olfactory bundles were subject to decomposition and their contents were subject to phagocytosis. Pictures of gliar proliferation were also observed. In all the stages studied (1-90 days) individual degeneratively changed mittal cells were found. Inflammatory processes in the olfactory nerve and in the olfactory bulbs finally died away. On the 30-50th day instead of the ejected elements there was seen an accumulation of gliar cells and an insignificant leucocytic infiltration. Thus, in our experiments we were faced with manifestations of a typical retrograde and transsynaptal degeneration ranging from the peripheral parts of the olfactory analyzer to its central links.

A different picture was seen upon damage of the olfactory bulbs (destruction with a sharp knife). The operation often led to hemorrhage at the site of the wound, as a result of which significant inflammatory manifestations appeared. The whole section sprouted with capillaries and was infiltrated with a large number of phagocytes. As a result in only 10-20 days the decomposing necrotic masses were resorbed and part of the capillaries growing before this wasted.

The inflammatory process in the region of the damaged olfactory bulbs of the brain was accompanied by an intense gliar proliferation, particularly distinct in the axolotl. Whereupon at the site of the destroyed sections of the olfactory bulbs characteristic gliar growths appeared having a gap similar to a rosette.

It was natural that the inflammatory process also spread to the region of the frontal brain which in the axolotl was unavoidably damaged during the operation. In this case some neurones degenerated and decomposed. The ependymal cells lining the cavity of the ventricles energetically multiplied by means of mitosis. Thanks to the drawing together of the internal walls of the ventricles they were able to eliminate the gap in the brain in these frontal sections. The edge was glued with a newly formed gliar scar, which led to deformation of the hemispheres; they became truncated or assumed another irregular shape. Proliferation of the ependyma was not accompanied by the appearance of neuroblasts in the reformation of the neurones either in the olfactory bulbs or in the investigated region of the frontal cerebral hemispheres. In this respect the results obtained conflict with a number of findings [3] regarding reformation of the neurones in the brain of low vertebrates on account of the cells of the ependymal lining in conditions of regeneration.

The degenerative changes in other parts of the frontal brain of the Crucian-carp and the axolotl, associated with the olfactory centers, were not followed up by us. As for the olfactory nerve and the olfactory sacs, i.e. the intermediary and peripheral parts of the olfactory analyzer, as a result of the damage inflicted in its central part, already on the 8-10th day pictures of degeneration and decomposition were seen in them. Changes in the olfactory lining on this occasion preceded changes in the olfactory nerve.

In the receptor layer of the olfactory sac at this time, on the 15-30th day after the operation, we found a considerable number of degenerated olfactory cells subject to necrobiosis. On the part of the supporting cells

we saw insignificant signs of proliferation. In the axolotl the Bowman glands were enlarged and showed signs of hypersecretion. In the olfactory nerve on the 8-10-15th day distinct pictures of degeneration were also present.

Thus, damage to the olfactory bulbs leading to destruction of the olfactory bundles and of the mitral neurones, produces a regular transsynaptal "response" on the part of the olfactory nerve and the olfactory receptor cells. If the whole bulb is destroyed, the olfactory cells on the entire length of the olfactory lining degenerate and disappear. It was not possible to establish reformation of the olfactory cells. On the other hand, multi-nuclear ciliated epithelium and supporting elements in such an olfactory lining proliferate mitotically.

Finally, one should note the following important factor. Upon damage of the left olfactory sac insignicant degenerative changes and gliar proliferation on the part of the neurones and of the glia of the right olfactory bulb and the olfactory and supporting cells of the right olfactory sac are observed. When the left olfactory bulb is damaged analogous changes are observed in the right bulb and the right sac. These facts demonstrate the trophic and probably the neuronal links in the paired olfactory organ.

## LITERATURE CITED

- [1] Aspiz, M. E. Trudy Severtsov Inst. Morfol. Zhiyot. 1954, 3, 92-113.
- [2] Budzilovskiya, L. G. Reactivity and Plasticity of Tissues (Leningrad, 1953), 16, 47-74.
- [3] Zelikina, T. I. Trudy Severtsov Inst. Morfol. Zhivot. 1954 11, 394-397.
- [4] Weissfeiller, J. Compt. rend Soc. biol. 91, 543-544 (1924).
- [5] Smith, C. G. Anat. Record, 109, 4, 661-669 (1951).

<sup>\*</sup> In Russian.