EFFECT OF STIMULATION ON REGENERATION OF THE SALIVARY GLANDS IN A COMPARATIVE SERIES OF MAMMALS

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Posttraumatic regeneration of the salivary glands was studied in rats, lambs, and dogs. Because of rapid maturation of the connective tissue forming a compact scar in the zone of resection, regenerative hypertrophy of the residual part of the organ was the predominant process. Administration of ethanolamine retarded maturation of the connective tissue. As a result, regenerative hypertrophy was activated and the growth of new tissues away from the wound surface was able to take place in the rats and lambs.

Despite much research into reparative regeneration of the major salivary glands in mammals [1-15], many of its aspects still remain incompletely studied or open to question (the method and completeness of regeneration, the presence of cambial elements, the fate and role of atypical lobules during regeneration, the relationship between the connective-tissue stroma and epithelial structures, the state of the vascular system and the nerves during regeneration, and so on). Regeneration of the major salivary glands in farm animals after resection has not been investigated, either under ordinary conditions or during stimulation.

The object of the present investigation was to compare the posttraumatic regeneration of the major salivary glands in albino rats, lambs, and dogs receiving ethanolamine as a stimulator of regeneration.

EXPERIMENTAL METHOD

The parotid and salivary glands of 500 albino rats (weight 100-120 g) and 60 lambs (age 4-5 months) and the parotid glands of 20 dogs (age 1-1.5 years) were investigated. Various operations were performed. In the case of unilateral resection, half the tissues of one gland were removed from the rats and from a quarter to a third of the tissues from lambs and dogs. To stimulate regeneration, some animals received subcutaneous injections of ethanolamine chloride in a dose of 1 mg/100 g live weight daily starting from the time of the operation. Where the observations continued for a long period, during the 6 days after the 5th injection the animals received a further 3 injections of ethanolamine. The animals were sacrificed daily during the 1st week, then at intervals of 2-3 days until 1 month, and thereafter 2, 3, 3.5, 5, and 9 months after the operation. Material was fixed in Bouin's and Baker's fluids, neutral formalin, acetone, etc. Serial paraffin sections were stained with hematoxylin-eosin, with picrofuchsin by Van Gieson's method, with Mayer's mucicarmine, and with toluidine blue and impregnated with silver by the Foot and Bielschowsky–Gros methods. Vitamin C was detected by the method of Giroud and Leblond, lipids by Sudan black, and the blood supply of the regnerating salivary glands were studied by intravital injection of the vessels with ink and gelatin.

EXPERIMENTAL RESULTS

On the day after resection of the salivary glands in these mammals, destructive processes developed in the central parts of the damaged lobules, leading to the development of necrosis. At the periphery of

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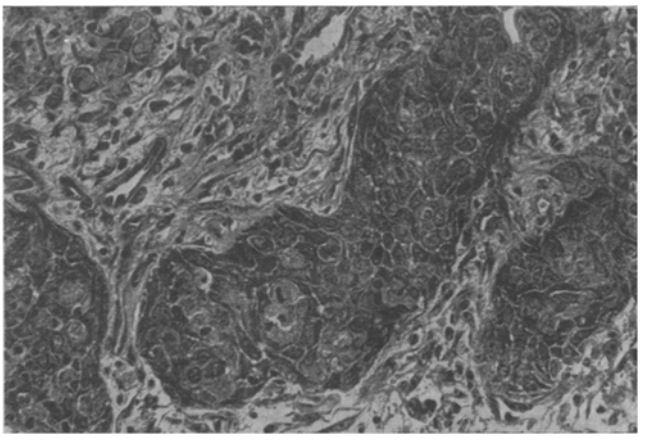

Fig. 1. Submandibular gland of a lamb. Stimulation with ethanolamine. Formation of epithelial bands on 6th day of regeneration. Van Gieson, 63 × 12.5.

these lobules in animals not stimulated with ethanolamine single acini and intralobular ducts still remained intact, and some of their cells were in an active state and undergoing mitotic division. In some of the damaged lobules destruction of the acini and ducts had occurred to a slight degree. Their epithelia was flattened, the cell boundaries were indistinct, and the lobules had acquired an atypical structure. Injection of the stimulator improved the chances of preservation of the damaged lobules (many more viable acini and ducts remained in the necrotic lobules), and in lambs' cells containing mucin appeared in the intermediate portions and salivary tubes, accompanied by intensive leukodiapedesis and considerable activation of mitosis both in the zone of resection and in parts of the gland distant from it.

On the 3rd-6th days after resection of the glands, necrosis in the central parts of the damaged lobules was intensified in the control animals. The efferent ducts in these parts were indistinguishable from the acini. All were merged into a general necrotic eosinophilic mass. Cells of the efferent ducts and acini remaining intact at the periphery of these lobules were undergoing mitotic division. As a result, their epithelial cover was stratified.

In the stimulated animals at this time, because of activation of elimination processes the necrotic focus was gradually being cleared of dead elements which were being replaced by granulation tissue. Meanwhile epithelial bands were formed from the residual peripheral acini and ducts of the damaged lobules, and these grew into the necrotic focus along the undifferentiated connective tissue (Fig. 1). In some intact lobules of the salivary glands close to the zone of resection, the circulation in the rats was severely disturbed, and the vessels were not filled with ink. Nerve fibers were not found or they consisted only of palely impregnated fragments. In these lobules the lumen of the acini and ducts was clearly defined because of a decrease in height of the epithelium, no granules of secretion were present, and the cell boundaries were only faintly visible. Considerable proliferation of the intralobular connective tissue was observed, so that the lobules were converted into atypical forms with obvious atrophy of the glandular structures. In areas of the gland close to and far from the zone of resection mitosis of the fibroblasts and glandular cells was activated and the diameter of the acini was increased. In lambs, for example, the diameter of the acini close to the regnerating zone of the parotid gland was increased on the average by 32%, while in distant lobules it was increased by 21%. In these places, besides typical acini, small acini with a well defined lumen also appeared.

On the 9th day of regeneration in animals not receiving ethanolamine proliferation of the epithelial bands was confined to the damaged lobules. In most damaged lobules, the glandular elements in the zone of resection were atrophied and replaced by connective tissue, forming a compact scar.

In the animals receiving ethanolamine, branching of the intra- and extralobular epithelial bands, terminating in nodular collections of cells, was observed at this time. The damaged lobules were thus partially restored and new ones were being formed. In the lambs and rats, the central cells in the terminal nodules and epithelial bands had become keratinized and were being desquamated, with the consequent appearance of a lumen (Fig. 2). The epithelial cells of these structures were stratified in their arrangement and

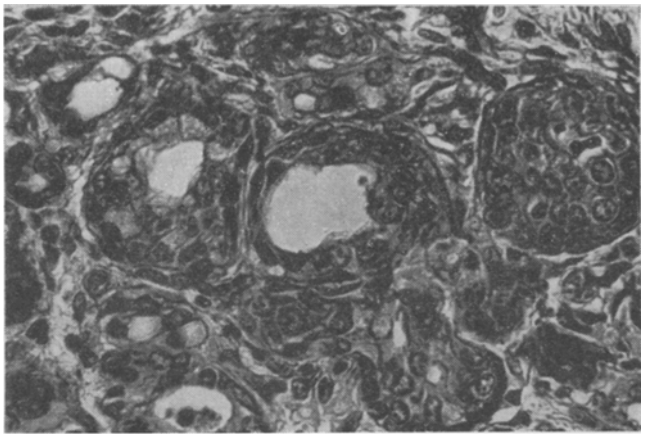

Fig. 2. Submandibular gland of a lamb. Stimulation with ethanolamine. Newly formed atypical lobule in zone of resection on 9th day of regeneration. Hematoxylin-eosin, 63 × 12.5.

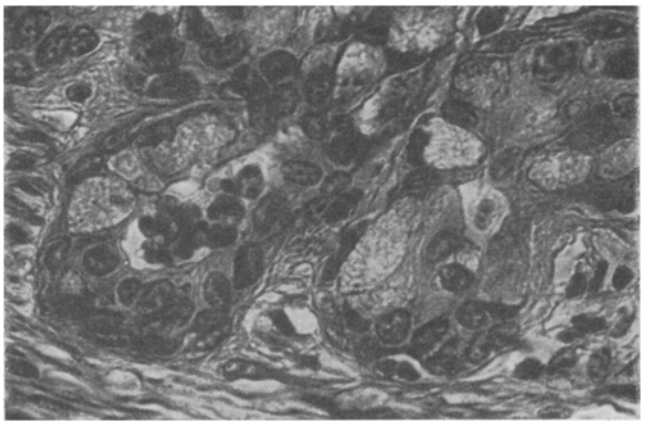

Fig. 3. Submandibular gland of a lamb. Stimulation with ethanolamine. Differentiation of newly formed secretory portions on 12th day of regeneration. Hematoxylin-eosin, 100 × 12.5.

there was no clear differentiation into glandular, covering, and myoepithelial cells. Mitoses were frequent. Numerous leukocytes were found in the lumen. They possibly played a role in the canalization of the epithelial bands and formation of a lumen. The connective tissue continued to remain undifferentiated. In this way the development of regenerative changes in the epithelial structures was facilitated for a comparatively long time, so that their connection with the undamaged parts of the gland could be established. In this way the conditions were created for secretion to be discharged and for further differentiation of the regenerating glandular structures to take place. In lambs, for example, among the newly formed acini of the submandibular gland, differentiation of mucous and serous cells could be observed (Fig. 3). In this way new lobules were formed and developed.

In the later stages of regeneration (5 months after resection in the rats, 9 months after resection in the sheep), numerous atypical lobules still remained in the zone of resection in the salivary glands of the unstimulated animals. Their acini were much smaller than in the neighboring intact lobules and they consisted mainly of mucous cells (the submandibular gland of sheep). On stimulation, both the regenerated and the newly formed acini were larger and had clearly defined mucous and serous portions. The great majority of atypical lobules had acquired the typical structure.

The course of regeneration of the parotid salivary gland in dogs, in which it is rich in connective tissue, was distinctive. In the animals undergoing resection, regeneration in the early periods followed

the usual course for sheep and rats. However, by the beginning of the 2nd week after resection, in both unstimulated and stimulated dogs intensive growth and rapid differentiation of the connective tissue were observed, so that it replaced the regenerating young epithelial structures.

The relationship between the glandular elements and connective-tissue stroma thus plays a decisive role in the development of regeneration of the salivary glands. Regeneration of glandular elements was suppressed by the progressive differentiation of the connective tissue. The use of ethanolamine as stimulator promoted the continued existence of connective tissue in an undifferentiated state for a long time, thus enabling a more normal regeneration of the glandular elements to take place. In this way, not only was regenerative hypertrophy stimulated, but growth of the tissues away from the wound surface was encouraged.

LITERATURE CITED

1. G. S. Arkhangel'skaya, in: Regeneration and Cell Division [in Russian], Moscow (1968), p. 7.
2. A. G. Babaeva, Byull. Éksperim. Biol. i Med., No. 3, 95 (1957).
3. A. G. Babaeva, in: Problems in Reparative and Physiological Regeneration [in Russian], Moscow (1960), p. 25.
4. A. G. Babaeva, in: Regeneration and Cell Division in Animals [in Russian], Moscow (1964), p. 78.
5. A. G. Babaeva, Uspekhi Sovr. Biol., 59, No. 2, 301 (1965).
6. E. Sh. Gerlovin, Trudy Leningrad. San.-Gig. Med. Inst., 42, 132 (1958).
7. E. Sh. Gerlovin, Development and Reactivity of the Major Salivary Glands in Ontogenesis. Doctoral dissertation, Leningrad (1961).
8. P. P. Gusak, A Study of Reparative Regeneration of the Major Salivary Glands. Candidate's dissertation, Leningrad (1966).
9. V. M. Koropov, The Pathological Physiology of the Salivary Glands [in Russian], Moscow (1949).
10. A. A. Ovsepyan, in: Proceedings of an Inter-institute Scientific Conference on Regeneration and Transplantation of Mammalian Organs and Tissues [in Russian], Erevan (1968), p. 60.
11. A. A. Ovsepyan, in: Proceedings of an Inter-institute Scientific Conference on Regeneration and Transplantation of Mammalian Organs and Tissues [in Russian], Erevan (1968), p. 62.
12. V. Podvissotzky, Beitr. Path. Anat., 2, 19 (1887).
13. A. Carraro, Frankfurt. Z. Path., 3, 26 (1909).
14. F. Fuckel, Über die Regeneration der Glandula Submaxillaris und Infraorbitalis beim Kaninchen, Freiburg im Breisgau (1896).
15. B. B. Milstein, Brit. J. Exp. Path., 31, 664 (1950).