REGENERATION IN THE SUBMAXILLARY GLAND OF THE RAT.

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THE mechanism of regeneration in salivary tissue has not been investigated since the researches of Podwyssozki (1887) and Ribbert (1894), who studied the problem in the rabbit. At that time, the tubulo-racemose structure of the submaxillary gland had not been described (Müller, 1895 ; Cohoe, 1907) so that the place of the tubular part of the gland in the regenerative process was not appreciated.

METHODS.

The experiments were performed on the submaxillary glands of male albino rats. The rats were selected for uniformity in body weight, and weighed either 200 or 250 g. approximately. Two experiments were performed, the first qualitative, the second quantitative. For the first experiment, the rats were divided into groups of six. They were operated on under open ether anaesthesia with full aseptic precautions. Through a midline incision in the neck, one submaxillary gland was exposed, and separated from the major sublingual gland, which is enclosed in the same capsule. About five-sixths of the gland was removed from half the animals in the group. The main vessels and duct were ligated with 43 S.W.G. stainless steel wire, but the cut surface of the gland, which bled only slightly, was not ligated. The other half of the group served as controls. These animals underwent mobilization of the gland, which was then allowed to fall back into its bed. Each group was killed at intervals between 1 day and 3 months after operation.

In the second experiment, three groups of 6 animals were used. One group was killed, and both glands weighed, the major sublingual glands being included. This provided the mean weight of one gland. The second group underwent removal of one gland and half the other, and were allowed to survive for 3 weeks. In this group the divided gland was cut across with scissors, and no ligature was used, haemorrhage being controlled by temporary pressure. The third group served as controls. These underwent mobilization of one gland and half the other, and were also killed after 3 weeks. The last two groups were pair-fed before and after operation in order to maintain their weights equal as far as possible. All animals were fed on M.R.C. rat cubes and water.

At the end of the experiments, the animals were killed with ether, and the remaining glands and portions of glands were dissected out and weighed. The animals in the first experiment received an intraperitoneal injection of colchicine (2 mg./kg.) 8 hours before death in order to arrest mitosis, and they were all

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killed at the same time of day. All the glands were fixed in 10 per cent formol-saline, and paraffin sections prepared for staining with Ehrlich's haematoxylin and eosin or Altmann's acid fuchsin and picric acid. The latter stain is useful for identifying the granules in the tubular portion of the gland.

In another group of 6 animals, the parotid, major sublingual and submaxillary glands were removed in two-stage operations, leaving only the minor sublingual glands as functioning salivary tissue. These rats were killed 3 months after the second operation, and the minor sublingual glands were dissected out and weighed. The tissues round the mouth and jaws were carefully examined for the presence of regenerated salivary tissue.

RESULTS.

Qualitative changes.—No macroscopic evidence of regenerated tissue could be seen. Microscopical examination of specimens removed one day after operation, showed marked degeneration. In some preparations all the acinar and duct cells in whole lobules had undergone cloudy swelling and fatty degeneration. In others, a fringe of normal acini persisted at the edges (Fig. 1). The cells which had not undergone degeneration appeared normal apart from an increased separation due to oedema. Contrary to the findings of Podwyssozki (1887) no mitoses were noted. Some of the smaller veins were thrombosed. After 3 days granulation tissue could be seen growing into the cut edge of the remaining portion of gland. The acini in the adjacent gland showed a moderate number of mitoses, but the most striking change was in the wall of the main duct. This was now 3 to 4 cells thick, instead of the normal single layer, and there were numerous mitoses amongst these cells (Fig. 2). A control gland which had been slightly damaged during mobilization also showed many acinar mitoses.

After 5 days the hyperplasia of the lining cells of the main ducts was still pronounced and mitoses were numerous. Branching ducts could now be seen growing into the granulation tissue along the cut edge, and in places these were proliferating to form acini. The process of formation of new acini from the old ones by division could still be seen in progress close to the line of section (Fig. 3). The nuclei of the new cells were larger than normal. The new acini were small and darker-staining than the mature ones, the cytoplasm staining deep red with eosin. Some of the new acini had a small lumen. The acini at the ends of the proliferating ducts were small, and composed of 2 to 4 cells. An early stage of duct proliferation is shown in Fig. 4. Areas in which new small acini were developing from ducts were sometimes seen in the normal gland away from the cut edge, and also in the controls, in a few cases (Fig. 7). These presumably represent compensatory hypertrophy or normal growth of the gland.

At the end of a week the proliferation of ducts and their differentiation into acini was still more marked and division of acini was taking place. One control gland at this stage showed duct proliferation with many mitoses in a small area at the edge in which haemorrhage had occurred during mobilization (Fig. 8).

After this time, both early and late stages of regeneration could be seen in the same sections. This is not surprising since regeneration would be expected to show first in the least damaged areas and later in areas which have suffered more degeneration.

At 2 weeks, what appeared to be a completely regenerated lobule of gland was seen in one preparation (Fig. 9). In a section stained with Altmann's acid

fuchsin, the tubular part of the gland could just be recognised in the new lobule in the form of occasional cells containing zymogen granules. The nuclei in the tubular part were fatter, paler and more numerous than normal and the granules in the cytoplasm less eosinophilic and smaller.

In one preparation obtained at 3 weeks, a newly formed gland composed of at least 6 lobules was seen (Fig. 11). There was a fringe of normal gland along one edge. The main duct was bent into a U-shape. The connective tissue in the new gland was not increased, i.e. the gland was formed entirely of new ducts and acini. The main ducts, faintly eosinophil, and lined with columnar epithelium, were breaking up into fine eosinophil ducts, lined by low columnar epithelium, which had much smaller nuclei. From these the acini budded off. The acini were at first dark red and solid. Later they enlarged, became round and had a small lumen. When they had grown to full size, they became irregular from the pressure of adjacent acini, and the lumen disappeared. The distal parts of the ducts at this stage had not developed cytoplasmic granules and still had the appearance of ducts and not that of the tubular part of the gland. This was confirmed with Altmann's stain. The bending of the duct seen in this preparation is the cause of obstructive changes which were seen in some specimens and which prevented regeneration in them.

Another regenerated lobe was seen in a specimen at 4 weeks (Fig. 12). This showed new acini in all stages of development. The younger ones were small and stained pink with eosin. They had relatively large but not very dense nuclei. The mature ones were larger, and their cytoplasm was slightly basophil. Occasional tubular areas could be distinguished in this specimen.

In later specimens complete regeneration was difficult to identify. Many apparently normal lobes were noted but these may have survived from the time of operation. Three features were found useful in determining whether a lobe had regenerated; separation of all the acini by oedema, crowding of the ducts suggesting that they had regrown parallel to one another, and absence or sparseness of the tubular part.

These features were all in evidence at 5, 6 and 8 weeks. In one 8-week specimen there were two newly-formed lobules, one of which possessed a tubular part. In this specimen also a long terminal duct showed acini budding off it (Fig. 14). Fig. 16 depicts a new lobule after ten weeks with its main duct entering. it. The acini still stain slightly darker than normal and the tubular part is only slightly developed.

Quantitative evidence of regeneration.—The results obtained are summarized in Table I. It will be seen that the submaxillary glands of normal 200 g. rats may be expected to increase in weight by 26·5 per cent in 3 weeks. The mean increase in weight of the portions of gland left after partial removal was approximately 22 per cent, suggesting that regeneration does not occur. But of the 6 animals used, the glands of 3 failed to show any increase in weight during the 3 week period of survival. The glands of the other 3 animals increased in weight by 33, 41 and 47 per cent, a significantly greater figure than was found in the controls. The reasons for this discrepancy will be discussed later.

When the animals which had lost all the salivary tissue except the minor sublingual glands were killed, no evidence of regeneration was apparent. The minor sublingual glands were not increased in size, and no other salivary tissue could be found. This suggests that there is no stimulus to regeneration of

TABLE I.

Group.	Number of rats in group.	Mean body wt. at operation (g.)	Mean wt. L. gland (g.)	Mean wt. part of R. gland removed (g.)	Mean wt. remaining R. gland (g.)	Mean body wt. 3 weeks after operation (g.)	Mean wt. R. gland 3 weeks after operation (g.)	Mean wt. L. gland 3 weeks after operation (g.)
1. Removal of L. gland and half of R. gland from 200 g. rats	6	207	210·8	112	98·8	248·7	121·5	..
2. Mobilization of L. gland and half of R. gland from 200 g. rats	6	208·5	..	..	..	257	245·4	233·8
3. Normal 200 g. rats	6	208·8	190	194		..	..	..

Body weight and weights of submaxillary glands before and after removal of one gland and half the other.

salivary tissue, for which the probable reason is that the saliva is not essential. However, it was noticed that when these animals were feeding, they found it necessary to take up a position beneath a water bottle and to drink copiously after each bite of a rat cube, presumably to moisten the food.

DISCUSSION.

Although definite quantitative evidence of regeneration in the submaxillary gland was not obtained, the microscopical findings leave no doubt that regeneration both of acini and ducts does occur, although it was not found in every gland examined. There are several reasons for this. Since five-sixths of the gland was removed, the remaining portion was very small. In spite of care in operating in order to avoid damage to the vessels and nerves, venous thrombosis was observed in some cases and the vascular damage may have resulted in ischaemia of the remaining portion of gland so that it could not regenerate. In other cases, the duct had become angulated, causing obstruction (Fig. 11). When obstruction occurred, the ductules dilated, and the acini disappeared, and finally infection of the duct supervened. Since it appears from this investigation that the most important sources of regeneration are the cut ends of the ducts, the amount of regeneration would depend partly on the number of ducts divided. In the first experiment, the gland was divided near the proximal end where its cross-section is small. The number of divided ducts from which regeneration could occur was therefore not as great as it would have been if the division had been performed at the site of the maximum cross-section.

The early changes found are similar to those described by Podwyssozki (1887), except that mitoses were not in evidence 24 hours after operation. Specimens were not examined at 48 hours, but mitoses were numerous after 3 days. Acinar mitoses were not found after 1 week, but the growth of newly formed ducts had commenced after 5 days. According to Podwyssozki (1887) these ducts mostly undergo a " regressive metamorphosis," with the formation of giant-cell conglomerations, or cysts and masses of fat droplets, only the smallest ducts being converted into new glandular acini. No regressive changes were found in my material and all the newly formed ducts appeared to develop acini at their terminations.

Ribbert (1894) was able to recognize naked-eye the regenerated areas after

removing half the submaxillary gland in the rabbit. The failure to do so in my experiment was due to the small size of the regenerated areas. The microscopical findings, however, are similar to those of Ribbert (1894), i.e. regeneration of acini occurs from the growth of large and small ducts. In my investigation it was possible to study regeneration in the tubular part of the gland. It was found that the tubular part did not appear to give rise to acini to any great extent or, if it did, the process was associated with a loss of the specific appearance and granules of the tubular cell so that it could not be distinguished from a large duct cell. Moreover, when a lobule regenerated, the regeneration of the tubular part was slow, slight and incomplete. Fig. 10 and 13 show new gland lobules stained with Altmann's acid fuchsin. The dark-staining areas are for the most part young acini, which take up the acid fuchsin, and not the fuchsinophil granules of the tubular part.

The small lumen in the new acini, on which Ribbert (1894) laid great stress as showing that the acini were newly-formed and had originated from ducts, was not seen in the earliest, dark-staining acini but only in slightly later, larger ones. The original acinar bud from the duct is solid, and the lumen only appears when the acinar cells have multiplied. It seems likely that it is not a remnant of the duct lumen, but a newly formed ductule, which is at first relatively large but rapidly narrows down to the normal size, when it is no longer visible.

The formation of new acini in the substance of the gland away from the cut edge may be regarded as compensatory hypertrophy rather than as regeneration. Ribbert (1894) concluded that the submaxillary gland of the rabbit showed only a slight tendency to hypertrophy, but this phenomenon occurred fairly often in the glands of my rats. It might be argued that this vitiates any positive results which were found in the quantitative experiment since the opposite gland was

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EXPLANATION OF PLATES.

FIG. 1.—Two lobules, one of which shows extensive cloudy swelling and fatty degeneration. 1 day. Haematoxylin and eosin. × 55.

FIG. 2.—Hyperplasia of lining cells of duct. 3 days. Haematoxylin and eosin. × 190.

FIG. 3.—Acinar mitoses. 5 days. Haematoxylin and eosin. × 535.

FIG. 4.—Early stage of duct proliferation. 5 days. Haematoxylin and eosin. × 250.

FIG. 5.—Newly-formed acini and ducts. 5 days. The new acini are above and to the right. Haematoxylin and eosin. × 190.

FIG. 6.—New acini forming from ducts. 5 days. Haematoxylin and eosin. × 250.

FIG. 7.—New acini forming away from cut edge. 5 days. Haematoxylin and eosin. × 220.

FIG. 8.—Control gland. Duct proliferation at edge in area of haemorrhage. 1 week. Haematoxylin and eosin. × 190.

FIG. 9.—New lobule. 2 weeks. Haematoxylin and eosin. × 40.

FIG. 10.—New lobule. 2 weeks. Altmann's acid fuchsin. The dark areas are young acini and not tubular parts. × 27.

FIG. 11.—New gland. 3 weeks. Fringe of normal gland above. The bending of the duct is clearly seen. Haematoxylin and eosin. × 18.

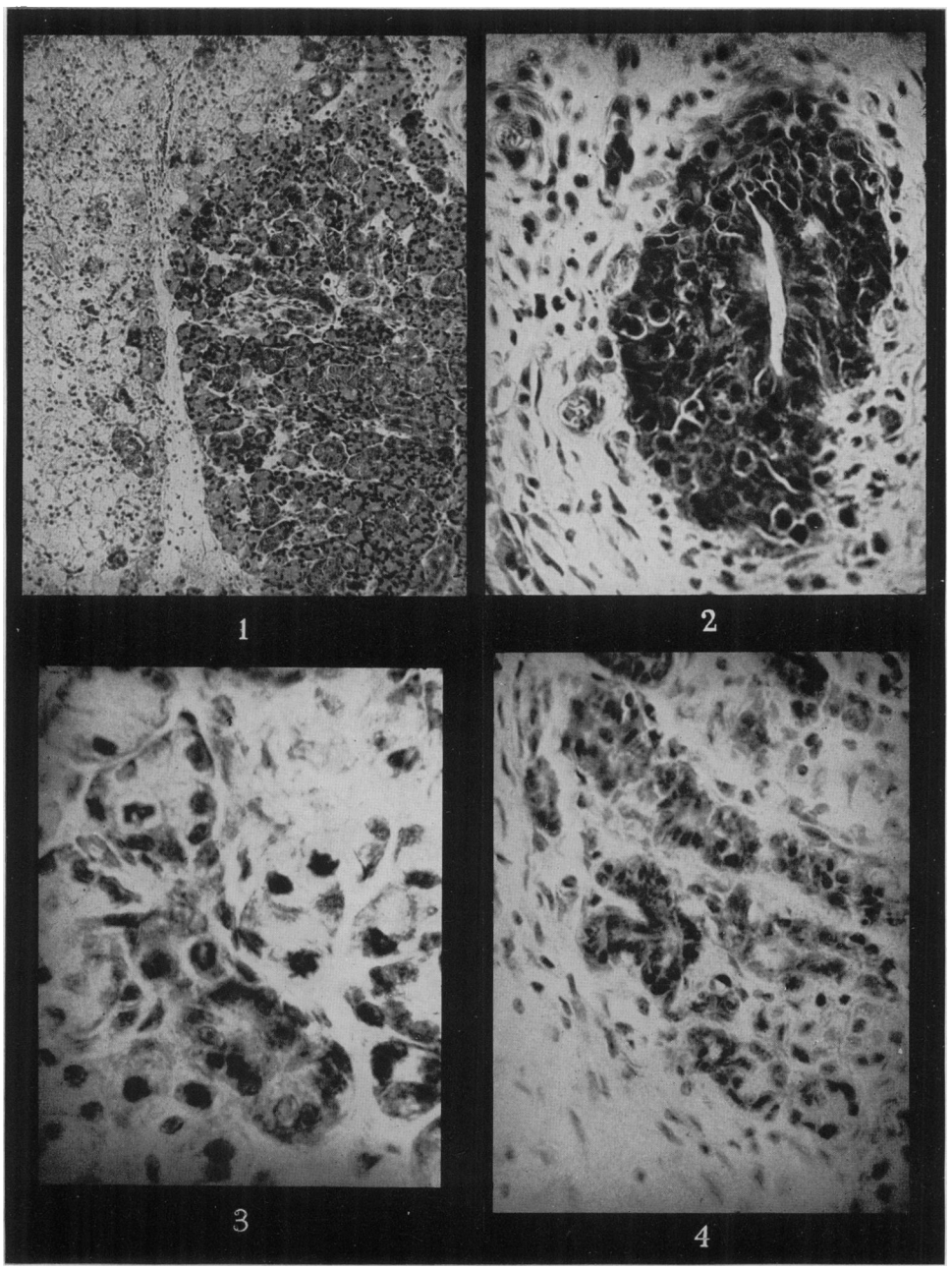
FIG. 12.—Regenerated lobule. 4 weeks. Haematoxylin and eosin. × 13.

FIG. 13.—Same as Fig. 12. Altmann's acid fuchsin. Note the sparseness of the tubular part. × 13.

FIG. 14.—Budding duct. 8 weeks. Haematoxylin and eosin. × 115.

FIG. 15.—New lobule. 8 weeks. Haematoxylin and eosin. × 78.

FIG. 16.—Duct entering new lobule. 10 weeks. Haematoxylin and eosin. × 25.



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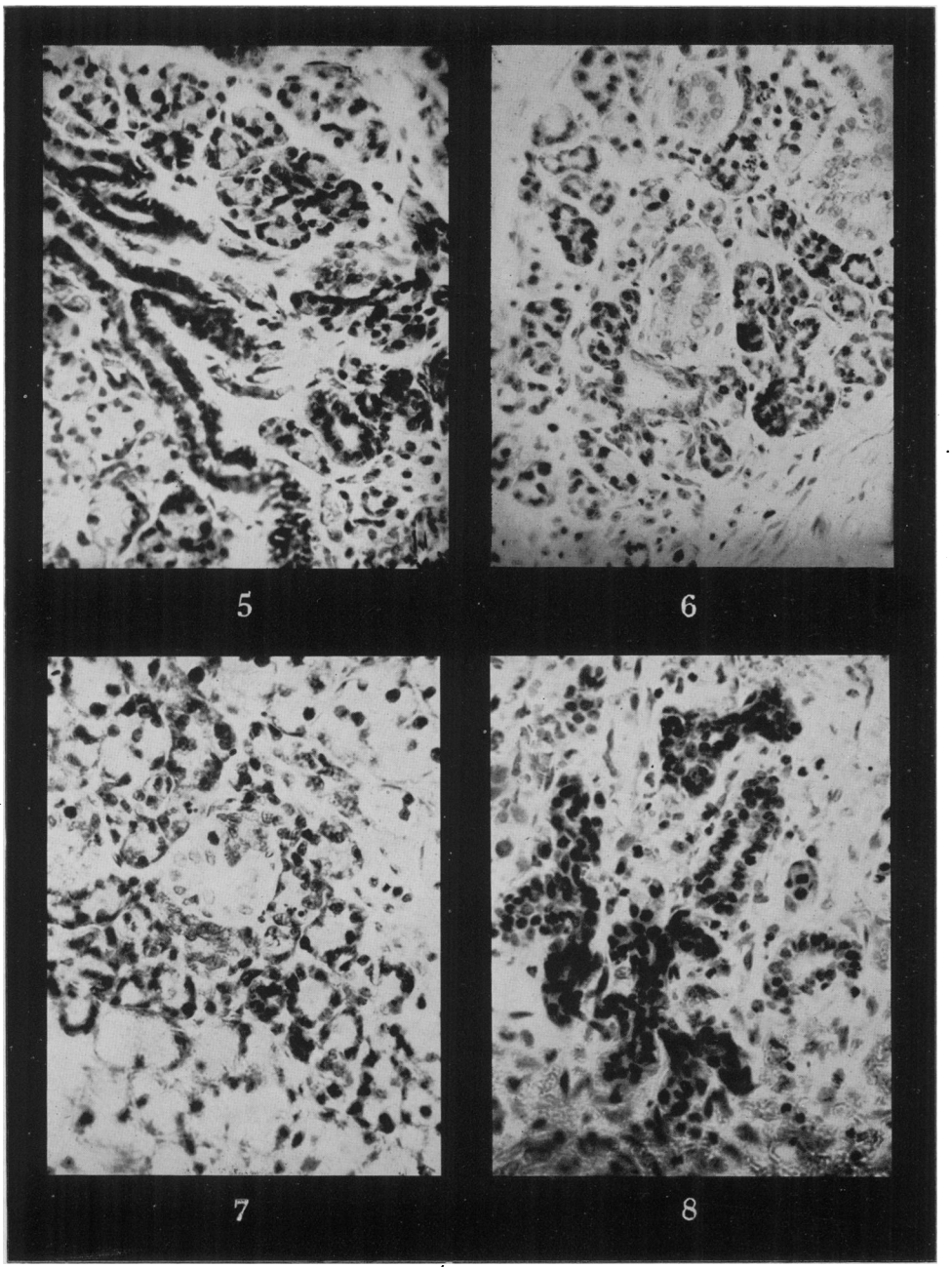

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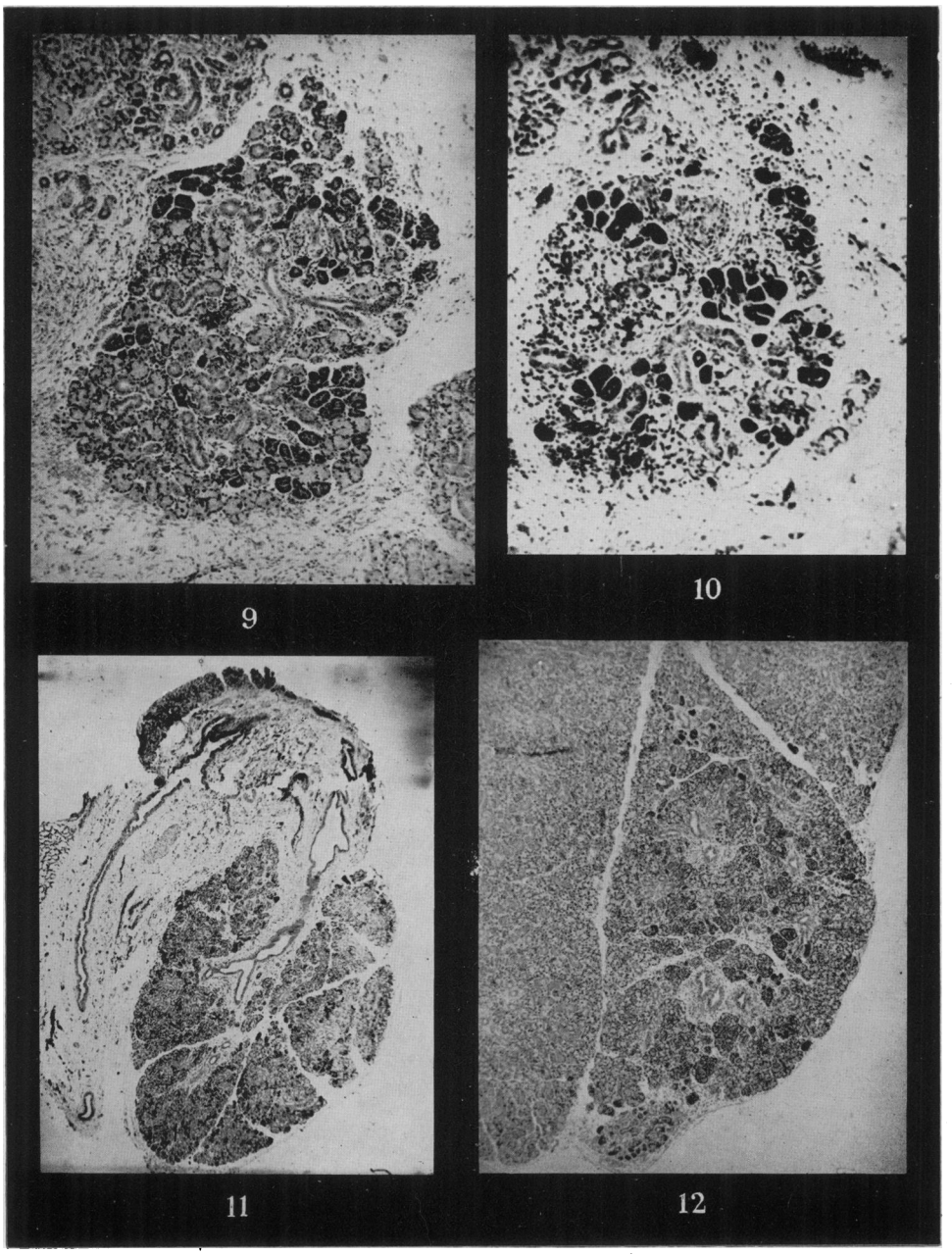

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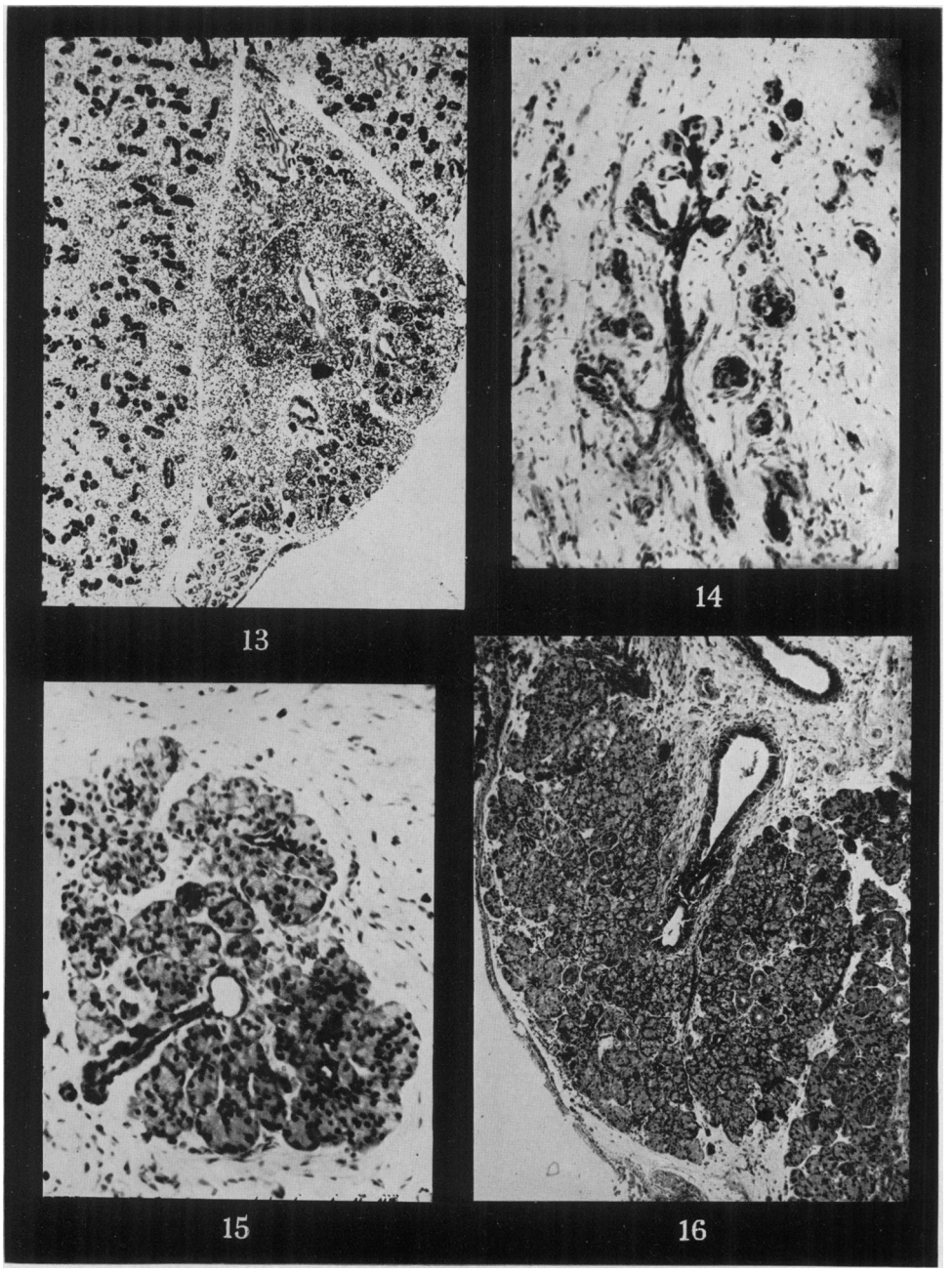

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removed. In fact, removal of glandular tissue does not appear to be a stimulus to hypertrophy, but injury to the gland does initiate regeneration, although there is no proof that the amount depends of the degree of injury. However, too fine a distinction between regeneration and compensatory hypertrophy cannot be drawn, since they both represent the same process.

The failure of regeneration after total extirpation of the major sublingual, submaxillary and parotid glands is surprising, but is in conformity with the findings of Schafer and Moore (1895–6) in the dog.

SUMMARY.

The mechanism of regeneration in the submaxillary gland of the rat after partial excision was investigated.

During the first week regeneration occurs as a result of division of pre-existing acini.

After the first week, ducts proliferate and branch in the granulation tissue and acini develop on their terminations.

The tubular part of the gland regenerates incompletely.

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