mucosa. The discs consist of 2 separate chambers. Both chambers are supplied with slender rubber tubes which pass out through the mouth. The disc is held in place by means of a slight amount of suction which can be applied by gently sucking the exposed end of the rubber tubing which is attached to the outer chamber. The secretion is free to flow through the inner chamber and push a bead of water through a calibrated capillary tube which is attached to the rubber tubing that leads out from the inner chamber. The secretory pressure of the inactivated as well as the activated secretion is adequate to move the bead of water through the capillary tubing.

The secretion from the oral mucosa is the most viscous of the salivary secretions. If the discs are removed with care the secretion adheres to the discs and may be collected for qualitative analyses. In some cases an hour may be necessary before a 0.05 cc sample of inactivated secretion can be obtained. Activated or stimulated secretions can be obtained more readily. The secretagogic activity of chewing dry bread serves adequately when larger samples are desired. In our studies on parotid secretion[6] we found the parotid secretory activity to be a sensitive index of organic changes. Although the glands in the oral mucosa have a similar neural innervation and are readily accessible, their excessive viscosity and slow rate of flow limit their use for this purpose. However, by means of the suction discs it is possible to collect samples of secretion which may be of some diagnostic aid.

These discs, which were originally developed by Lashley can also be used in some cases as a substitute for cannulation in the study of other glandular activities in animals.

10327

Rôle of Capsule in Suprarenal Regeneration Studied With Aid of Colchicine.

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All the successful functional transplants reported in the literature seem to depend upon the creation of an initial suprarenal insufficiency, as originally postulated by Halsted.[1] Such studies have been

[6] Winsor, A. L., and Korchin, B., *J. Exp. Psychol.*, 1938, **23**, 62.

[1] Halsted, *J. Exp. Med.*, 1909, **11**, 175.

made by Jaffe and Plavska,[2] Blodinger, Klebanoff and Laurens,[3] Higgins and Ingle,[4] Wyman and tum Suden,[5] Ingle and Higgins,[6] and Baker and Baillif.[7]

The study herewith reported is an endeavor to locate the sites from which regeneration takes place in the enucleated suprarenal gland, and to follow the process of regeneration from the time of transplantation until the completion of the regenerative process. A recent study along somewhat the same lines by Zwemer, Wotton, and Norkus[8] also concerns itself with the histology of the normal adrenal cortex and the origin of new and removal of old corticoadrenal cells under experimental conditions.

Suprarenal insufficiency was produced in 37 immature and mature male and female albino rats by the removal of the right gland and the enucleation of the left, according to the procedure devised by Ingle and Higgins.[6] In a few instances the capsule and residual cortex were transplanted in the abdominal wall. All animals survived the operation.

The usual laboratory diet was employed, and no cortin or sodium chloride was added to it. The animals were sacrificed at daily intervals from the first to the tenth day, and at weekly intervals thereafter to the fifty-seventh day.

Because the site of most active cell proliferation during the process of regeneration is marked by numerous mitoses, we endeavored to make this phenomenon more evident by the use of colchicine, which, with a few exceptions, was given subcutaneously 9.5 hr before the animals were sacrificed.

The tissue to be examined was fixed in Bouin's fluid and serially sectioned. The glands were stained with Heidenhain's azan mixture and Krichesky's modification of Mallory's triple connective tissue stain.

Within the first 3 postoperative days the capsule becomes edematous, hyperemic and thickened. By the 2nd day it has lost its dense, heavily-staining appearance and becomes more loosely constructed. The cells and their nuclei, especially in the inner half of the capsule, become oval, and many begin to undergo mitosis. Prominent blood vessels appear among these transforming cells.

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2 Jaffe and Plavska, PROC. SOC. EXP. BIOL. AND MED., 1926, **23**, 528.

3 Blodinger, Klebanoff and Laurens, PROC. SOC. EXP. BIOL. AND MED., 1926, **23**, 22.

4 Higgins and Ingle, *Proc. Staff Meet. Mayo Clinic*, 1937, **12**, 69.

5 Wyman and tum Suden, *Endocrinology*, 1937, **21**, 523.

6 Ingle and Higgins, *Endocrinology*, 1938, **22**, 458.

7 Baker and Baillif, *Anat. Rec. Suppl.*, 1938, **70**, 5.

8 Zwemer, Wotton and Norkus, *Anat. Rec.*, 1938, **72**, 249.

The changes described in the capsular cells (Fig. 1) tend to produce a stratified appearance in the capsule as regeneration progresses. The innermost sheets of fibers become broken up, due to a centripetal migration of the former capsular cells. The process is probably not one of active movement but rather a change in the mitotic planes of the ingrowing cells. In early capsular hyperplasia the plane of mitosis is parallel to the inner surface of the capsule. As the process proceeds, the plane changes through an angle of 90° and the multiplying cells, as a result, begin to proliferate inward from the capsule. Later they fill the space left by removal of the inner portion of the gland. The cortex is thus replaced by cells derived from the substance of the capsule.

The newly formed cortical cell has a relatively large nucleus and a very small amount of cytoplasm. The nucleus is at first slightly fusiform, but very soon becomes oval. Then it increases rapidly in size and becomes round as the new cell pushes its way into the glomerular zone. The quantity of cytoplasm increases slowly.

The cells within the capsule which are being transformed into cortical cells begin to show a small amount of lipoid in their cytoplasm. The lipoid appears first as tiny droplets at one side of the nucleus, in the region of the centrosphere. As they increase in size, the droplets spread throughout the cytoplasm. In the course of cytological transformation the early cortical cell increases markedly in size, becomes round, and accumulates more and more lipoid. These changes are usually more marked after the cells pass the inner margin of the capsule, but may be seen while they are still enclosed by capsular fibers. Under these conditions an island of cortical cells arises within the capsule. The most active stage of cortical regeneration occurs between the second and seventh days.

Concomitant with the ingrowth of transforming cortical cells, a second cytological element is seen to proliferate from the capsule in the form of a small, stellate cell, which is apparently actively migrating inward. This cell appears after the process of cortical hyperplasia has begun, but soon outstrips the glandular cells in migration. It very rarely shows any stainable lipoid in its cytoplasm. This element is destined to supply the connective tissue support for the ingrowing glandular cells and blood vessels.

The cortical zones are seen to regenerate in the following manner:

a. Narrow cords of cells appear as ingrowths from the capsule, giving rise, by further ingrowth and multiplication, to the cell groups which are destined to become the zona glomerulosa.

b. From the inner margin of these cell groups, directed by the

radially arranged connective tissue strands, new cell cords proliferate toward the center of the gland to form the zona fasciculata.

c. The inner ends of these cords show branchings among the connective tissue in the center of the gland, and a zona reticularis is seen after 3 weeks.

The tissue left *in situ* after enucleation of the gland is a mere shell

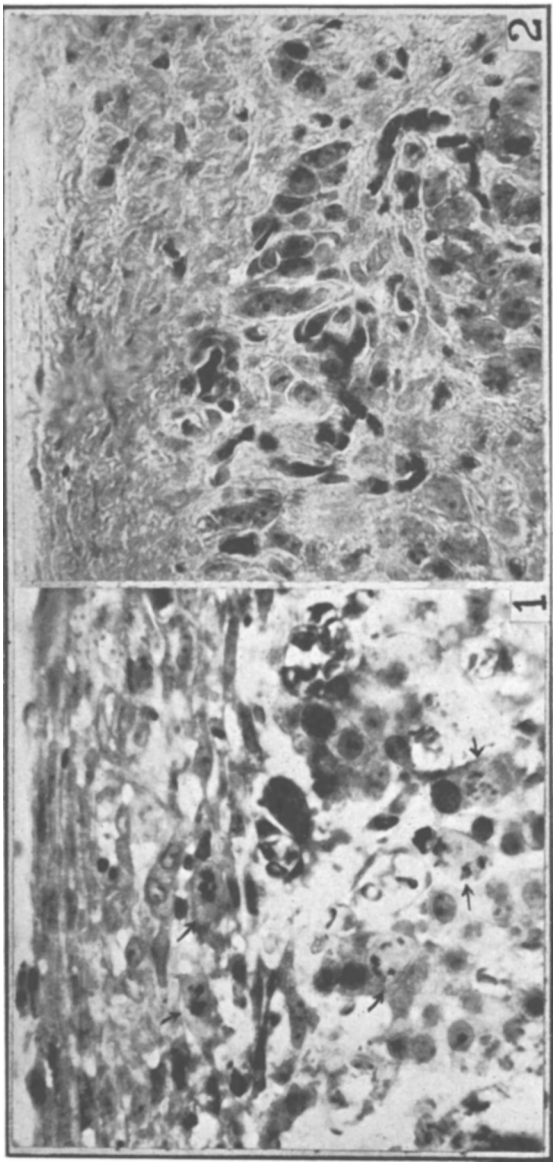

FIG. 2.
Few mitotic cells are seen without the use of colchicine. × 525.

FIG. 1.
Mitotic cells are more frequently seen in the inner than in the outer half of the capsule. Colchicine. × 525. Some mitoses indicated by arrows.

which promptly becomes edematous and filled with a blood clot. This clot becomes organized rapidly and is canalized by ingrowth of the capsular connective tissue and blood vessels. This process is followed by a gradual resorption of the clot, beginning at the periphery, which allows space for ingrowth of the regenerating cortical cells.

Small but definitely marked islands of medullary tissue were noted in 4 regenerating glands removed after the 20th day. In 3 of these the islands are seen embedded in the edge of the central fibrous tissue mass, and in the 4th several small medullary islands are seen in the scar of the operative incision. These cells are readily distinguished from the adjacent cortical cells by their arrangement into pseudofollicular groups separated by a connective tissue stroma. It was also noted that they have a much more lightly staining cytoplasm than is characteristic of the cortical cells. It is quite possible that these are medullary cells which remained attached to nerve bundles and were not removed when the gland was enucleated, for nerve trunks can be traced to them through the capsule and cortex.

The actual process of regeneration of the suprarenal cortex seems to be practically completed within 30 days. Since no signs of suprarenal deficiency were noted in any of the animals, it may be assumed that the enucleated gland begins to function within a few days after operation. No operative deaths occurred, but younger animals seemed to withstand the procedure better than fully matured ones. Our observations reveal no noticeable difference between the sexes in the rate of regeneration.

Mitotic cells were much more numerous in the regenerating glands of animals receiving colchicine (Fig. 1) than in those that did not (Fig. 2). The ratio of mitotic cells before and after giving colchicine was 1 :4.6.

Summary. Cortical cells and their supporting tissues regenerate from the capsule of the enucleated suprarenal gland. Mitotic cells are more frequently seen in the inner half of the capsule. These new cells become oval and migrate toward the center of the regenerating gland, some to form cortical cells and others to form supportive tissue for the suprarenal cords. The cytoplasm of the new cortical cells very early contains lipoid, but that of the supportive cells contains no fatty substances. The central blood clot becomes organized and canalized.

In 4 glands nerves could be traced through the capsule to groups of medullary cells. The ratio of mitotic cells before and after the administration of colchicine was 1 :4.6.