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Regeneration of Rat Tracheal Epithelium After Mechanical Injury. I. The Relationship Between Mitotic Activity and Cellular Differentiation[1,2] (37968)

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(Introduced by A. C. Upton)

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Respiratory epithelium is composed of a relatively slowly renewing cell population (1) whose turnover rate is markedly increased by injury and consequent cell death (2). In order to assess whether control of mitosis depends on the relative numbers or arrangement of mature amitotic cells, we mechanically damaged the mucosal surface of rat tracheas to eliminate a proportion of the mature cells and examined the temporal relationship between replenishment of epithelial cell numbers and restoration of specialized cytologic structure. The appearance of goblet cell and ciliated cell differentiation in the regenerating cells was found to follow the peak of mitotic activity. These results suggest that control of cell division in this epithelium is not dependent on a property of the fully differentiated cell population.

Materials and Method. One hundred and ten female Sprague–Dawley rats, varying in weight from 125 to 175 g, were lightly anesthetized with Diabutal administered by intraperitoneal injection. The anterior surface of the trachea was exposed by sharp dissection and retraction and incised with a scalpel through the third cartilage ring. A blunt probe was inserted and lightly stroked against the dorsal surface of the trachea. In injuring the epithelium, we have taken care to avoid denuding the surface, which has been previously reported to result in a delay in mitotic response until cells at the margin of the lesion migrate to cover the exposed connective tissue (3, 4). The tissues were then apposed and the skin closed with two steel clips. Sterile technique was employed and a single operator performed each group of experiments. Postoperatively, morbidity and mortality was minimized by keeping the rats in a humidified recovery chamber at 30° until they were able to right themselves. Animals were reanesthetized with Diabutal and their tracheas removed at 2, 12, 18, 24, 28, 30, 32, 36, 44, 48, 60, 72, and 90 hr after the original surgery. Tracheas from at least 3 animals were examined for each time point. In addition, tissue from animals anesthetized but not subjected to surgery was examined immediately and at 2, 12, 36, and 60 hr to assess effects of anesthesia, and the trachea from at least one animal from each group of 24 animals was excised immediately after injury to determine the extent and nature of the damage.

For most time points, two or more additional animals received an intraperitoneal dose of colchicine given at a dose of 1 mg/kg body wt 3 hr prior to sacrifice. This served to arrest mitosis and thus facilitate recognition of periods of peak mitotic activity.

At the time of sacrifice, the trachea was excised in its entirety and fixed by intraluminal perfusion with and immersion in

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4% glutaraldehyde buffered to pH 7.4 with 0.2 *M* sodium cacodylate. After a period of 24 hr, during which the tissues were hardened by fixation, the trachea was cut into 2–3 mm wide rings, treated with 1% unbuffered osmium tetroxide, dehydrated with graded alcohols, and embedded in Epon 812. Survey sections including the entire mucosal surface of each ring in the areas of injury were examined with a light microscope and the discreteness of the lesion assessed. Rings from above and below the injured areas were also sectioned and served as an internal control for operative variables other than anesthesia effects. Thin sections for electron microscopic examina-

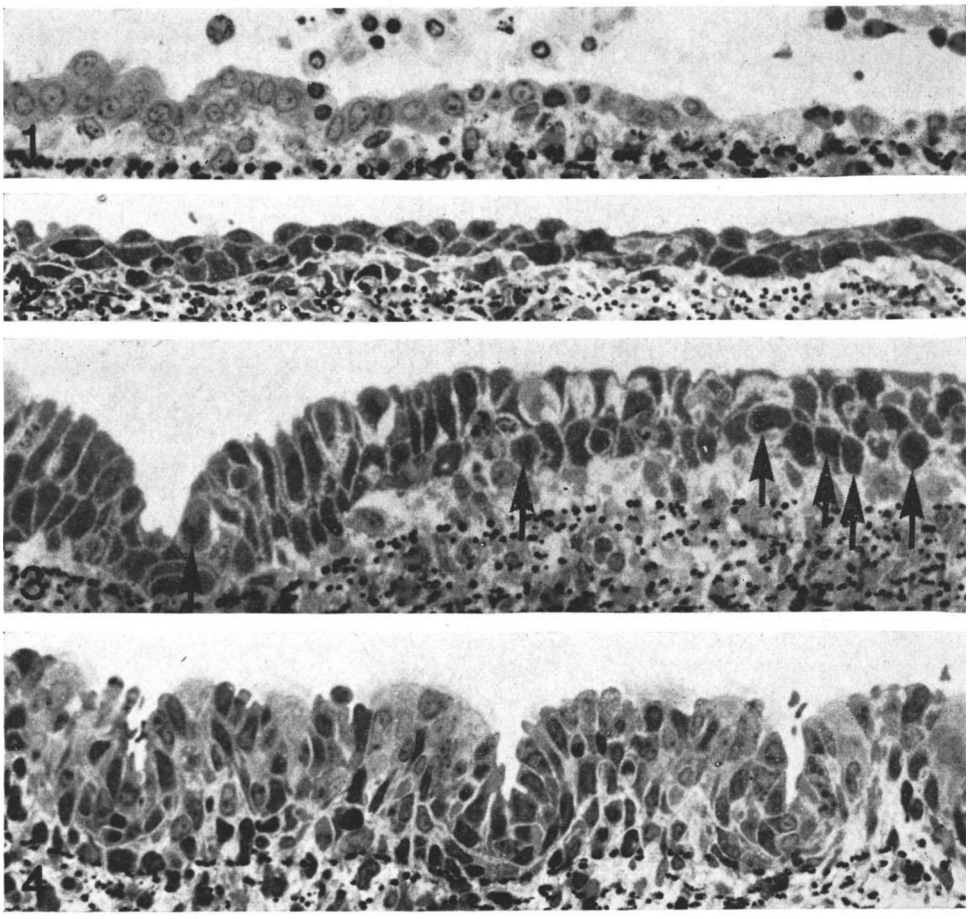

FIG. 1. Light micrograph of tracheal epithelium 2 hr after mechanical injury shows a focally discontinuous layer of residual ovoid basal cells. Stained with methylene blue and azur II. (×350)

FIG. 2. At 18 hr after injury, the tracheal epithelium consists of two layers of flattened cells lacking apical morphologic differentiation. Stained with methylene blue and azur II. (×350)

FIG. 3. The injured area of tracheal epithelium from an animal treated with colchicine at a dose of 1 mg/kg body wt at 29 hr after injury and sacrificed 3 hr later demonstrates basal and supra basal dividing cells (arrows). Other cells in the field lack apical morphologic differentiation. Stained with methylene blue and azur II. (×350)

FIG. 4. By 90 hr after injury, there is a return to normal pseudostratified epithelium. The surface cells exhibit typical apical morphological differentiation. Stained with methylene blue and azur II. (×350)

tion were prepared from the areas of injury and from the zones above and below the injury. Survey electron micrographs including the entire thickness of the epithelium were obtained from a representative section from each block sectioned and additional micrographs at higher magnifications were then taken to evaluate details of ultrastructure.

Results. Light microscopy. Tracheas fixed immediately after blunt mechanical injury of the dorsal mucosal surface demonstrated damage to most cells reaching the free surface. These cells were vacuolated, hydropic, or completely disrupted in light microscopic sections. Basal cells showed no changes. Tracheas from animals anesthetized but not subjected to mechanical injury showed none of these changes nor did cellular alterations appear in the control animals sacrificed at later time points.

At 2 hr, there was loss of most of the damaged cells in the area of trauma, leaving a single discontinuous layer of basal cells with an occasional residual ciliated or goblet cell (Fig. 1). By 12 hr, the single cell layer had become more regular and specimens obtained from 18 to 24 hr after injury revealed 2–3 layers of small cells similar to basal cells (Fig. 2). The tracheal epithelium from animals sacrificed 28–60 hr after injury showed three or more cell layers with some elongation of cells but without a true pseudostratified organization (Fig. 3). By

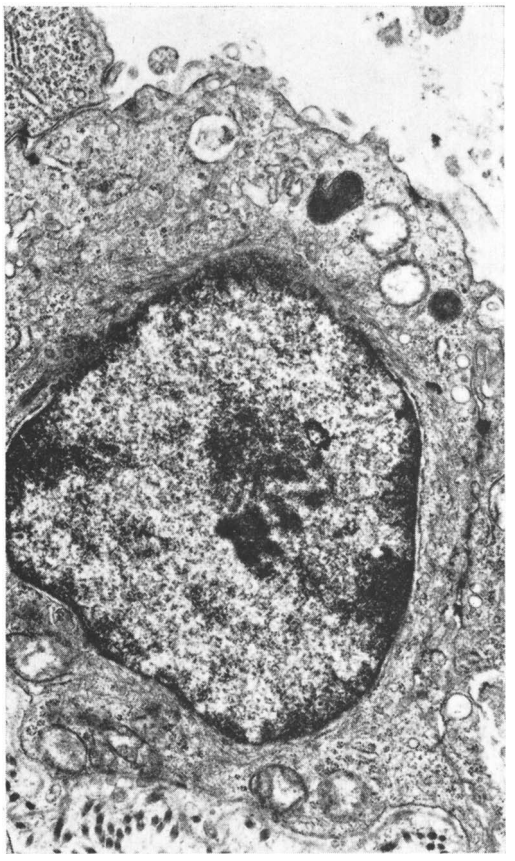

FIG. 5. Electron micrograph of tracheal epithelium 2 hr after mechanical surface injury reveals a single layer of intact cells resting on basement lamina. Supranuclear cytoplasmic differentiation is absent in the ribosome-rich cytoplasm. Stained with lead citrate and uranyl acetate. (×10,000)

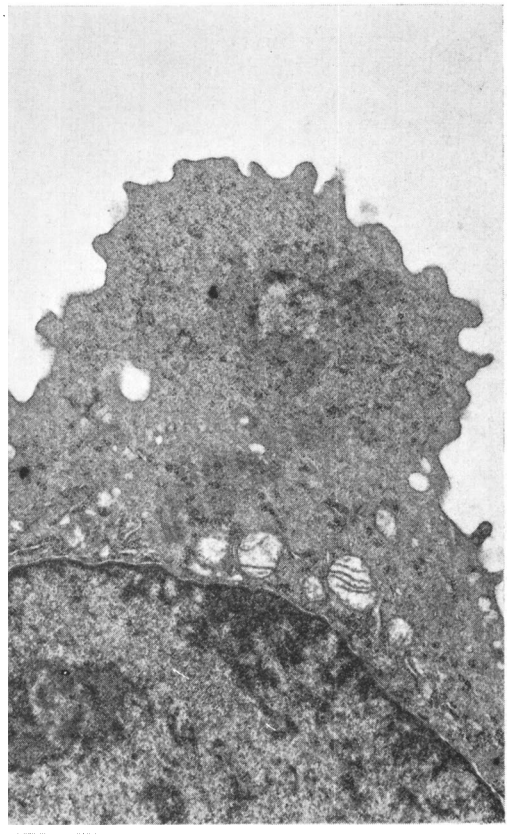

FIG. 6. Electron micrograph of apical surface of a superficial tracheal epithelial cell 30 hr after injury still lacks morphologic differentiation. Stained with lead citrate and uranyl acetate. (×10,000)

90 hr, the epithelium was composed of basal cells, goblet cells, ciliated cells, and intermediate tall cells in a somewhat cellular but clearly pseudostratified array (Fig. 4).

Mitotic figures were seen in all areas of damage in specimens obtained from animals treated with colchicine within 48 hr of injury. The mitotic count was greatest in specimens from animals receiving colchicine from 26 to 30 hr after injury (Fig. 3), but even in the 2-hr specimen, it was considerably above the very low background values in epithelium outside of the areas of injury.

Electron microscopy. The residual cells at 2 hr had the ribosome-rich cytoplasm and indented nucleus seen in basal cells and lacked free surface modifications (Fig. 5). Only an occasional cell with remnants of cilia or cytoplasmic specialization consisting of an abundance of granular endoplasmic reticulum and Golgi apparatus was seen in the area of injury and there were only small areas in which the basal lamina was denuded of cells. By 12 hr, gaps between cells were no longer encountered, and in the 18-, 24-, and 28-hr specimens, the polyribosome-rich cells were heaped on one another to form a double layer (Fig. 6). At 30, 32, 36, 44, and 48 hr, the basal-type cells became progressively more heaped so that they appeared to form three or more layers (Fig. 7). Some of the cells were columnar at 48 hr. These exhibited ovoid or rounded nuclei with extensive Golgi apparatus but contained few or no secretory

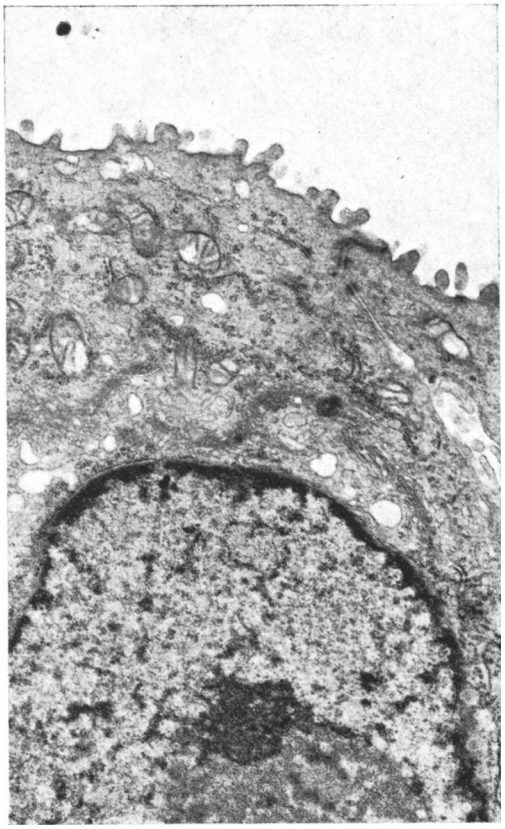

FIG. 7. At 44 hr after injury, this typical cell is from a uniform population of relatively undifferentiated cells. Although mucinogen droplets are lacking, there are increased numbers of mitochondria and golgi complexes. Stained with lead citrate and uranyl acetate. (×10,000)

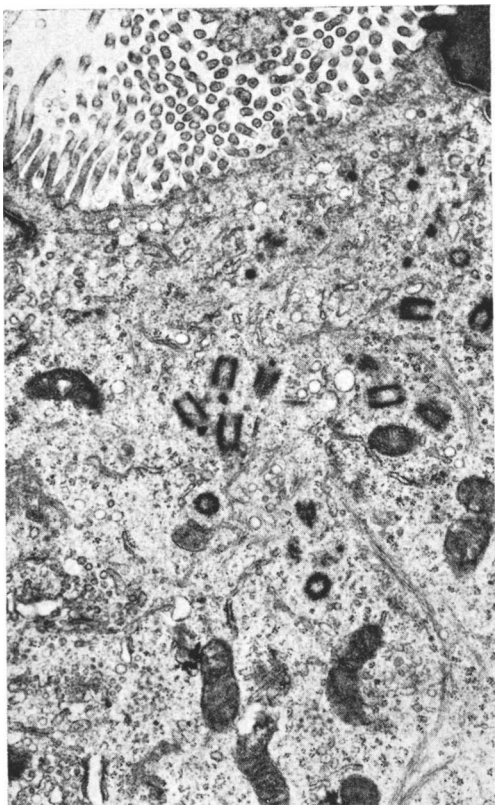

FIG. 8. At 60 hr after injury, the epithelium contains elongated cells of which many contain numerous nonpolarized basal bodies deep in the cytoplasm and apparently migrating to the apical surface. Stained with lead citrate and uranyl acetate. (×10,000)

granules. By 60 hr, groups of basal bodies could be discerned in some cells while others contained mucinogen droplets (Figs. 8, 9). The 90-hr specimen had crowded tall cells many of which had well-developed cilia or typical goblet cell morphology.

Discussion. Regeneration is a term that has been used to refer to several different kinds of restitutive processes. In skeletal muscle, where mitosis is uncommon, increase in tissue mass after minor injury consists primarily of cellular repair (5), and, after major injury, of expansion of a small dormant myoblast population (6). In liver and kidney, regeneration has been used as the label for a compensatory hyperplasia within functional units of residual tissue (7). The cells capable of division in these tissues have been described as "reverting post-mitotics" because no cell population which is specialized for mitotic activity and not for other specific tissue function can be identified in unstimulated tissue. Finally, the blood cell-forming tissues and the surface epithelia belong to a third group, the constantly renewing tissues which, when reduced to smaller cell numbers by injury, can regenerate by division of recognizable and ever-present basal or stem cells to reestablish normal numbers of basal and mature amitotic cells in their usual histologic organization (8).

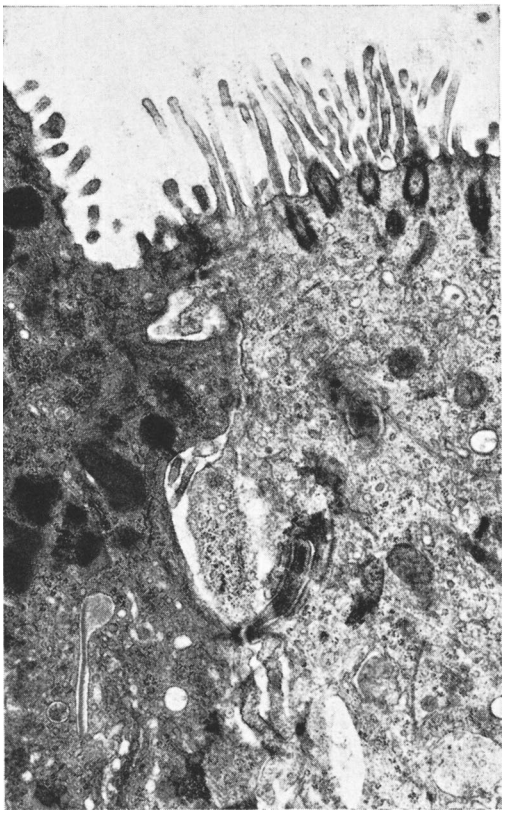

FIG. 9. By 60 hr basal bodies have reached the free surface in some ciliated cells and some goblet cells contain secretory granules. Stained with lead citrate and uranyl acetate. (×10,000)

It could be argued that distinguishing between these three groups of tissues is simply recognizing a greater capacity for cellular resistance to injury in muscle and other tissues in which basal cell division does not form the primary response to most injuries. This argument could also be made for liver, kidney, and other so-called "reverting post-mitotic" tissues with the additional observation that division occurs in defined regions of the hepatic lobule (9) and the nephron (10), suggesting that there may be a zone populated by cryptic basal cells which have the capacity to carry on other functions beside mitosis (11). It remains clear, however, that without injury there is a great difference between these tissues, which are characterized by low mitotic activity, and the constantly renewing surface epithelia. The process of regeneration in surface epithelia appears to be an acceleration or exaggeration of the normal mechanism for control of cell numbers in uninjured surface epithelia, while the mitotic responses in the two other groups of tissues are expressions of latent capacity. This would make a regenerating surface epithelium a better model for studying mitotic control in normal tissues.

Evidence in several tissues supports the hypothesis that it is a product or property of mature cells which suppresses division of potentially mitotic cells (12). A corollary of the postulate is that the loss of mature cells reduces the suppression of mitosis and the ensuing division serves to restore the number of mature cells. This reciprocity would thus act as a feedback type of control of division. We have found in this one type of regenerating epithelium that mitotic activity peaks before most features of the

mature cells are seen in the daughter cells. If there is a factor in mature cells which suppresses mitosis, our data shows it to be independent of some of the cytoplasmic specializations which we recognize as hallmarks of the mature end cell.

We also found that there was a period during which the cells were heaped up in several layers rather than pseudostratified as is normal respiratory epithelium. This regenerative hyperplasia is seen in other surface epithelia and a response proportional to the stimulation is also seen in liver, following surgical removal of various fractions of its original mass (13). The transient hyperplasia we observed in tracheal epithelium may reflect a massive response to an unbridled stimulus which results in excessive division before repression is reestablished. In the normal tissue, with the stimulus at a much lower level, there would be less mitotic response without an obvious pendulum effect in cell numbers.

Summary. The time of appearance of cytologic specialization in cells of regenerating rat tracheal epithelium was compared to the time of maximal mitotic response. Trauma sufficient to lethally injure most cells reaching the surface but sparing most basal cells resulted in a peak of mitotic activity from the 26th to the 30th hour following injury. The cells in the hyperplastic regenerating epithelium at the peak of mitosis had high nuclear–cytoplasmic ratios with little cytoplasmic or surface specialization. Ciliogenesis and mucinogen formation were not seen in most cells until the 60th hr after the injury and there was little cell division at that time or later. Since cessation of mitosis does not follow or even coincide with the replenishment of the ciliated or goblet cell populations, the data does not support the hypothesis that control of cell division resides in the elaboration or release of a suppressor substance by the mature cells.

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