Squamous Metaplasia of the Opening of Bronchial Glands

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The major bronchi of swine, a dog and rabbits were examined with a scanning electron microscope and the fine structure of the openings of bronchial glands were studied three-dimensionally. The smallest areas of squamous metaplasia involved the duct openings. By examining serial sections with the light and transmission electron microscopes these early lesions were found localized at the opening of the bronchial gland duct. Cells intermediate to goblet and squamous cells were present in these lesions. Squamous metaplasia starting at this particular location is probably common and metaplasia can be an intracellular process. (Am J Pathol 67:571–582, 1972).

ALTHOUGH SQUAMOUS METAPLASIA of the bronchus occurs most commonly in cigarette smokers, the lesion is also found in nonsmokers and children.[1,2,3] Squamous metaplasia may be found anywhere in the tracheobronchial tree as a result of local tissue reaction to injury. However, there are sites of predilection where metaplasia occurs more frequently for specific reasons. For example, squamous metaplasia is very common at the bifurcation of the bronchi [1,4] and has been explained by the heavier deposition of inhaled particles at this site, resulting in intense and prolonged alteration of the function of the mucociliary apparatus.[5] It has also been suggested that cephalad streaming of mucus results in concentration of deposited materials as the cross-sectional area of bronchial tree decreases. The concentrated mucus also appears to linger at bifurcations.[6]

Since squamous metaplasia occurs in the duct of exocrine glands, such as salivary glands or pancreas,[7] it seemed probable that it would commonly occur in the ducts of bronchial glands. However, squamous metaplasia of the bronchus is usually extensive when first seen and its origin is difficult to determine using the light microscope. We have found that the scanning electron microscope is very useful in detecting minute, and presumably early, lesions of the bronchial mucosa which frequently involve the ducts of bronchial glands. This report

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presents correlated findings of early squamous metaplasia in some mammals using the scanning electron microscope (SEM), the transmission electron microscope (TEM) and the light microscope (LM).

Materials and Methods

Major bronchi were obtained from normal young animals, including 2 swine, 2 New Zealand rabbits and a dog. The bronchi were fixed for 3 to 4 hours in 3.5% glutaraldehyde in 0.025 M sodium veronate buffer at pH 7.3. For scanning electron microscopy, the specimens were cut in 1 sq cm blocks with one flat surface being mucosa. The mucous membrane was thoroughly washed with normal saline and thick mucous was removed by mucolytic agents (Airbron, BDH Toronto or Sputolysin, Calbiochem. Los Angeles, Calif). The tissues were then postfixed in 1% osmium tetroxide, washed and dehydrated with graded acetone. The bronchi were air-dried with 100% acetone, coated with 200 Å thick gold-palladium and examined with a Cambridge Stereoscan scanning electron microscope. For transmission electron and light microscopy, appropriate areas containing the openings of ducts were cut into 1 mm blocks, postfixed in 1% osmium tetroxide and embedded in Epon. Serial sections were obtained and stained with 0.5% toluidine blue for LM or with uranium acetate and lead citrate for TEM. Some of the large specimens which had been examined with the SEM were reimmersed in acetone and embedded in Epon for LM and TEM examination.

Results

The Normal Opening of Bronchial Glands

If the surface of the bronchus was thoroughly cleaned, the opening sites of bronchial ducts could be identified by ordinary dissecting microscope. With the SEM the openings were seen more easily; in swine they formed rows, usually at the bottom of the longitudinal mucosal folds of the bronchus (Figure 1). However, in the dog and rabbit, the openings were irregularly arranged. At higher magnification, the openings of the bronchial glands appeared as depressions on the mucosal surface covered by cilia (Figure 2). In serial sections for light microscopy, several ciliated cells were present in the proximal part of the duct adjacent to its opening into the lumen (Figure 3). The lining cells of the duct beyond this (subsequently referred to as the neck of the duct) were low columnar or cuboidal cells usually containing serous granules but occasionally mucous granules. Oncocytes were not seen in our animals. The basal cells formed a continuous epithelial cell layer basal to the bronchial mucosa and the duct.

The ciliated cells in the proximal portion of the duct were identical to those in the bronchial mucosa as seen by TEM. The cells were tall columnar with cilia of up to 6 μ in length. The nuclei were usually ovoid and basal in position. There were supranuclear aggregations

of mitochrondria and many cytosomes. The lining cells of the duct showed well-developed microvilli, Golgi areas and abundant supranuclear secreting granules that distinguished the mucous and serous cells. Small numbers of tonofilaments were found beneath the mucous granules of the goblet cells. The basal cells of the duct were usually cuboidal or slightly flattened, and contained a round or ovoid nucleus and a few organelles. Characteristically, the basal cells contained bundles of tonofilaments, some of which were associated with desmosomes. Half desmosomes were also present in the basal region, and the basement membrane was well developed.

Squamous Metaplasia of the Openings of Bronchial Glands

With the SEM, one area of squamous metaplasia is usually found in each specimen. The opening of the bronchial duct was frequently, but not invariably, involved. Lesions, sharply limited in extent, with only a few metaplastic cells around the bronchial glands, were repeatedly seen in all animals (Figure 4). Serial sections through the minute metaplastic foci and the normal ductal openings revealed changes, which were arbitrarily classified into three stages, according to their severity and duration.

The Minimal Stage

The goblet cells were focally increased in number at the neck portion of the duct. The goblet cells were rich in mucous granules and were flask-shaped (Figure 5).

The Intermediate Stage

The goblet cells at the neck of the duct contained few mucous granules (Figure 6); the granules were of irregular sizes and were randomly scattered through the cell, and were even found in the subnuclear and basal region. Some mucous granules were shrunken and had homogeneous dense cores. These cells showed many clubbed microvilli on the luminal surface and many desmosomes between cells (Figure 7). Irregular bundles of tonofilaments, some of which were attached to desmosomes, were a striking feature of the cytoplasm; some of the altered goblet cells were so loaded with tonofilaments that a portion of the cell appeared as a mature squamous cell, while the other portion still retained mucous granules (Figures 8, 9).

Another type of cell showed many clubbed microvilli on its luminal surface and contained large numbers of tonofilaments in the cytoplasm; this type of cell did not have mucous granules but was rich

in small vacuolar structures in the cytoplasm (Figure 10). These cells were often found among the altered goblet cells mentioned above. At this stage, the bronchial ductal lumen was slightly dilated and plugged with inspissated material.

The Advanced Stage

Squamous cells appeared at the opening of the duct, and spread out to involve the adjacent bronchial mucosal surface (Figure 11). The cells were cuboidal or flattened and showed prominent microvilli at the luminal surface. The nucleus was ovoid and the cytoplasm was diffusely occupied by fine tonofilaments (Figure 12); some nuclei showed deep indentation. There was an increased layering of these epithelial cells. Mitotic figures were occasionally seen. The basal cell layer appeared unaltered. The squamous metaplasia extended down to the duct for some distance and there was an accompanying ductal dilatation and mucous plugging.

Discussion

The SEM produces three-dimensional surface pictures of tissue at high magnification and is therefore very useful in studying an organ with many surfaces (such as lung), especially when it is combined with the TEM.[8] In this study the openings of the ducts of bronchial glands were clearly shown with the SEM and the smallest areas of squamous metaplasia were localized at this site.

Studies of the openings of the bronchial ducts using serial sections with the LM and the TEM revealed three stages of change which may be sequential. It appeared that proliferation of goblet cells was the earliest response to injury, as shown by others.[5,6] In the intermediate stage two types of cell were found. Both showed clubbed microvilli and increased tonofilaments in the cytoplasm; residual mucous granules were found in some, and small vacuoles in others. The latter type of cell fits the description of "brush cells" of Rhodin[9] and could be the supporting cells.[10] The maturation of the nonkeratinizing squamous cells is characterized by an increase in number and diffuseness of tonofilaments in the cytoplasm.[11] This process of squamous maturation appeared focally in the cytoplasm of the altered goblet cells; some portions of the cell were squamous while other portions still retained mucous granules (squamoid goblet cell). Metaplasia is defined as a replacement of an adult cell by another adult cell type; the usual concept is that basal reserve cells differentiate to form adult cell types different from those normally present.[7] Squamous metaplasia in the bronchial mucosa is thus the replacement of ciliated or goblet cells

by squamous cells. Our observations of squamoid goblet cells suggest a transformation of goblet cells into squamous cells through a quantitative change of cytoplasmic components rather than a qualitative change in cells. This is not too surprising, since tonofilaments, the characteristic component of a squamous cell, are normally present in small amounts in the goblet cell.[12] It is less clear at what level of differentiation the change occurs.

Basal cells, rather than altered mature goblet cells, may be the source of these altered "squamoid goblet cells"; however, proliferation of basal cells was not observed. Furthermore, many cells with varying degrees of "squamoid goblet features" were present at the same site suggesting that the process is continuous and changing. Cytokinetic study of these cells will be helpful in resolving this problem.

Squamous metaplasia of the bronchial epithelium associated with specific conditions, such as tuberculosis, bronchiectasis, etc, can occur without involving the bronchial glands; however, squamous metaplasia starting at the openings of bronchial glands is probably very common for anatomical and pathophysiological reasons. The opening of the duct is very small and is normally surrounded by mucosal epithelial cells which form a relatively rigid structure. By contrast, the duct proper is surrounded by loose lamina propria, and the basal cells of the duct are not contractile. Normally the myoepithelial cells of the secretory acini contract in response to stimuli and propel the secretory material towards the duct and the opening. When the secretory material becomes viscid the opening site of the duct is subjected to physical stress, due to the narrowness of the opening and the relatively rigid surrounding structures. The duct dilates on these occasions, and the degree of dilatation appears to correlate well with the degree of squamous metaplasia at the site of the opening.

Although there were a few atypical cells with irregularly indented nuclei and prominent nucleoli, the lesions we observed were essentially benign. How far these lesions can progress towards atypia is uncertain. It is unlikely that squamous metaplasia due to simple stagnation of secretion progresses to a malignant lesion, since the lesions are common and bronchial carcinoma very rare in the species studied. However, the bronchial gland is known to concentrate injected dyes and excrete them via the duct; hence local concentration and stagnation of irritants or carcinogens could be important in carcinogenesis.

No oncocytes were observed in the ducts of bronchial glands. It thus seems unlikely that oncocytes are a normal component of the duct with a specific function in the animals we have studied, as has been suggested for oncocytes found in bronchial gland ducts in man.[13]

References

1. Knudtson KP: The pathologic effects of smoking tobacco on the trachea and bronchial mucosa. Am J Clin Pathol 33:310–317, 1960
2. Sanderud K: Squamous metaplasia of the respiratory tract epithelium. An autopsy study of 214 cases. I. Incidence, age and sex distribution. Acta Pathol Microbiol Scand 42:247–264, 1958
3. Auerbach O, Stout AP, Hammond EC, Garfinkel L: Changes in bronchial epithelium in relation to cigarette smoking and in relation to lung cancer. N Engl J Med 265:253–267, 1961
4. Auerbach O, Stout AP, Hammond EC, Garfinkel L: Bronchial epithelium in former smokers, N Engl J Med 267:119–125, 1962
5. Kotin P: Carcinogenesis of the lung: environment and host factors. The Lung. Edited by AA Liebow, DE Smith. Baltimore, The Williams and Wilkins Co, 1968, pp 203–225
6. Kotin P, Courington D, Falk HL: Pathogenesis of cancer in a ciliated mucus secreting epithelium. Am Rev Resp Dis 93:(3) 115–133, 1966
7. Robbins SL: Abnormalities of cell growth, Pathology, Third edition. Philadelphia and London, W.B. Saunders Company, 1967, pp 74–87
8. Wang NS, Thurlbeck VM: Scanning electron microscopy of the lung. Hum Pathol, 1:227–231, 1970
9. Rhodin JAG: Ultrastructure of the tracheal ciliated mucosa in rat and man. Ann Otol 68:964–974, 1959
10. Krahl VE: Anatomy of the mammalian lung, Respiration, Vol 1, Handbook of Physiology, Section 3. Edited by WO Fenn, H Rahn. Washington, DC, American Physiological Society, 1964, pp 213–284
11. Zelickson AS: Normal human keratinization processes as demonstrated by electron microscopy. J Invest Dermatol 37:369–379, 1961
12. Rhodin JAG: The ciliated cell: ultrastructure and function of the human tracheal mucosa. Am Rev Resp Dis 93:(3) 1–15, 1966
13. Meyrick B, Sturgess JM, Reid L: A reconstruction of the duct system and secretory tubules of the human bronchial submucosal gland. Thorax 24:729–736, 1969

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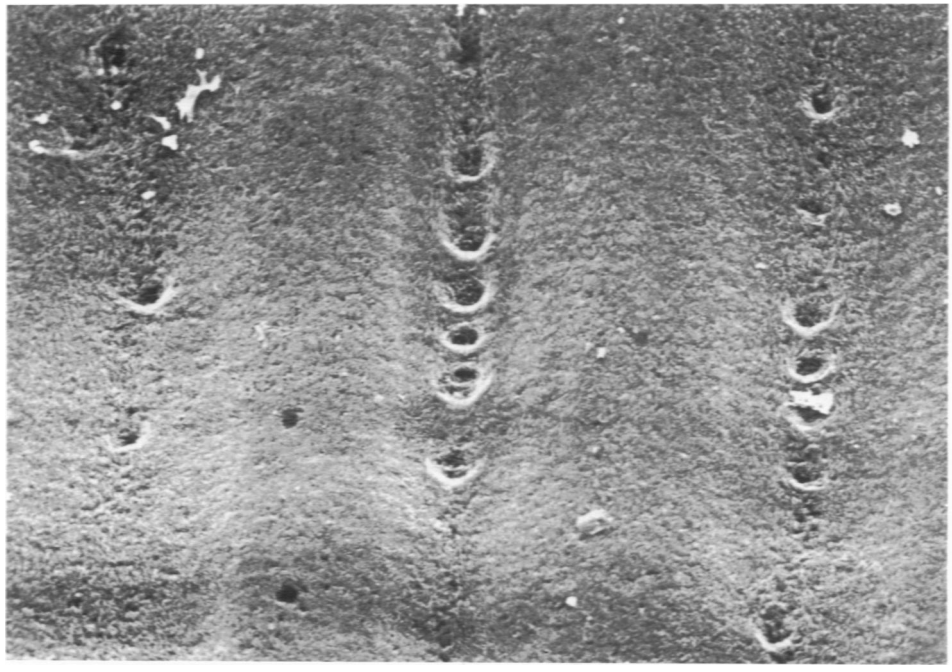
Legends for Figures

NOTE:

TBS: *toluidine blue stained 1 μ thick Epon section.*
TEM: *thin Epon sections, stained with both uranium acetate and lead citrate; pictures taken with transmission electron microscope.*
SEM: *pictures taken with a scanning electron microscope. Specimens coated with 200 A thick gold-palladium.*



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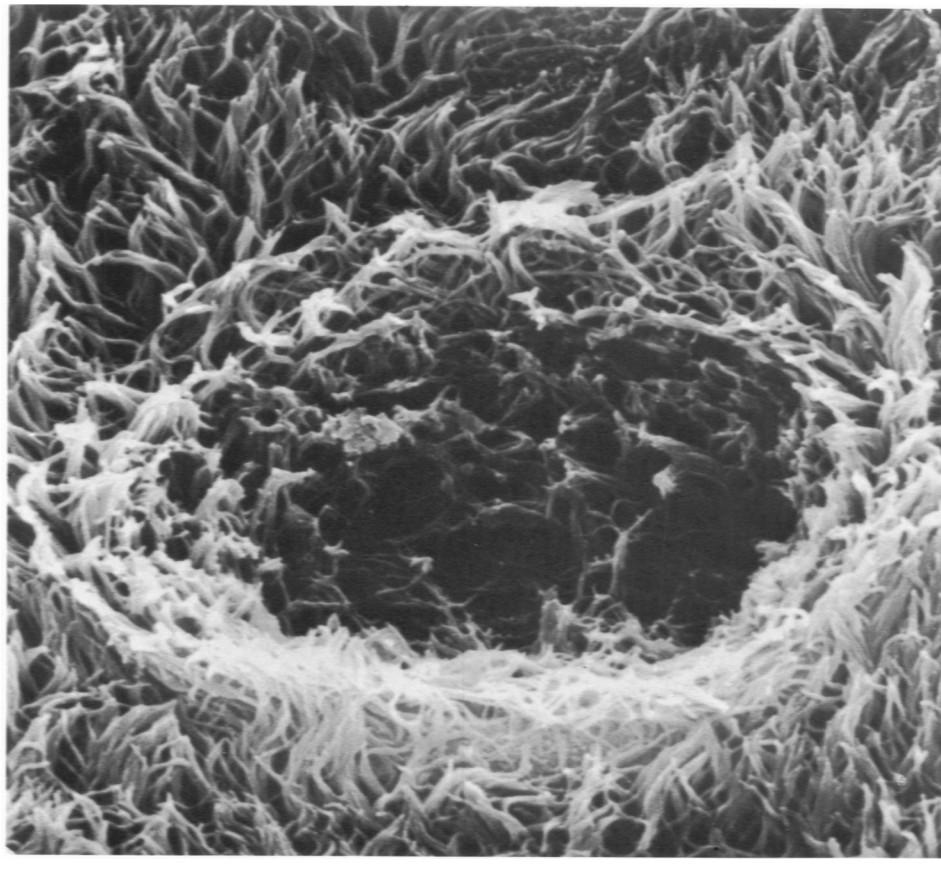

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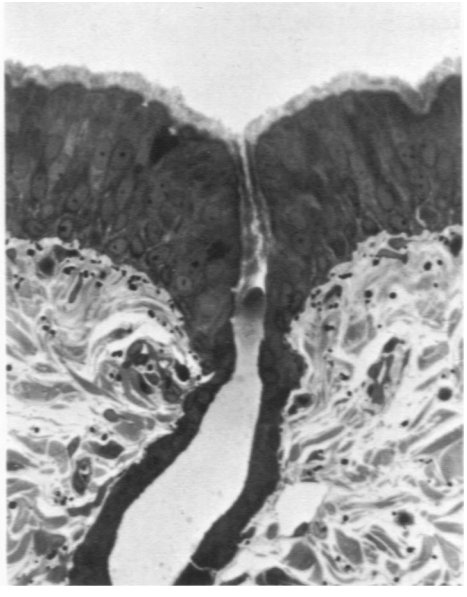
Fig 1—With the scanning electron microscope, openings of bronchial glands appear as pits and form regular rows at the bottom of the longitudinal folds on the bronchial mucosa (Swine SEM, × 250). **Fig 2**—Cilia over the bronchial surface and also the depressed pit-like opening of the duct of the bronchial glands (Swine SEM, × 5000).



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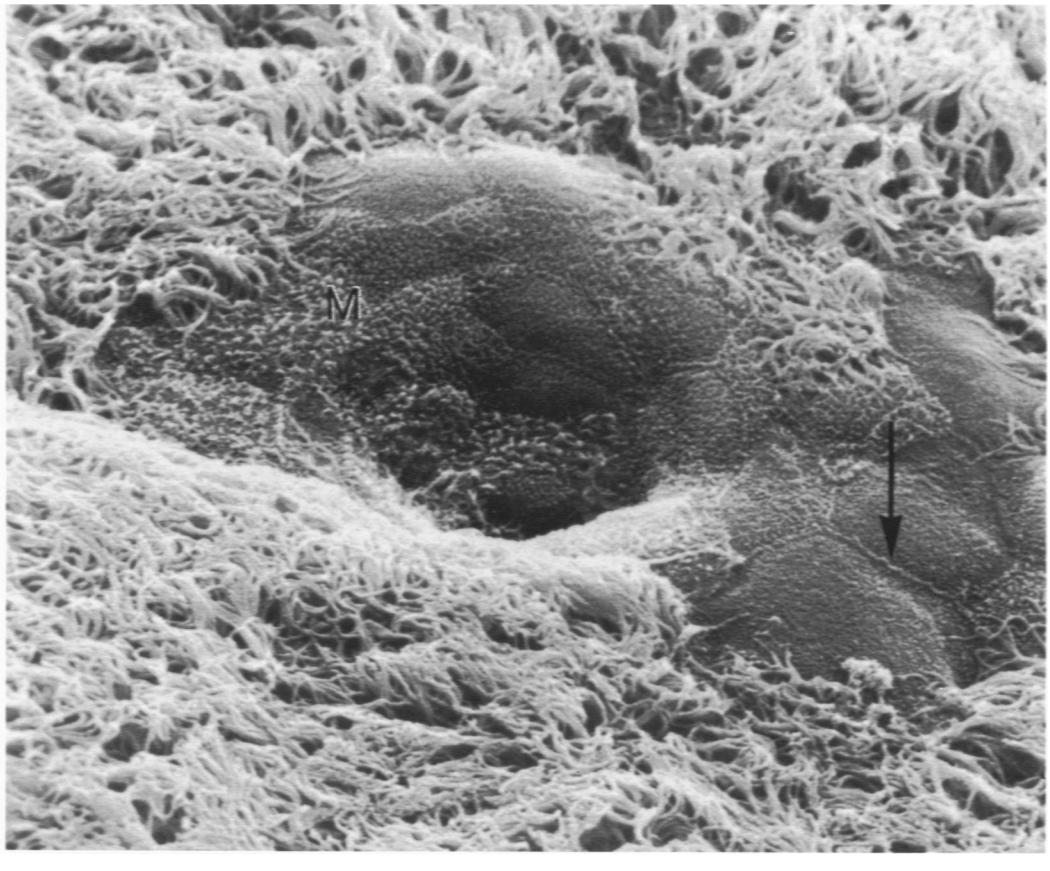

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Fig 3—Cross section of a pit-like depression (Figure 2) shows a normal opening of a bronchial gland. Only a few ciliated cells extend into the duct (Dog TBS, × 400).

Fig 4—An opening of a bronchial gland showing a squamous metaplasia. The lesion bulges out and is sharply defined from the surrounding ciliated cells. Cell boundaries (*arrow*) and microvilli (*M*) are clearly shown (Swine SEM, × 5000).

Fig 5—A minimal lesion. Goblet cells (*arrow*) accumulate around the opening of the bronchial gland. Most of the goblet cells appear normal (Swine TBS, × 300).

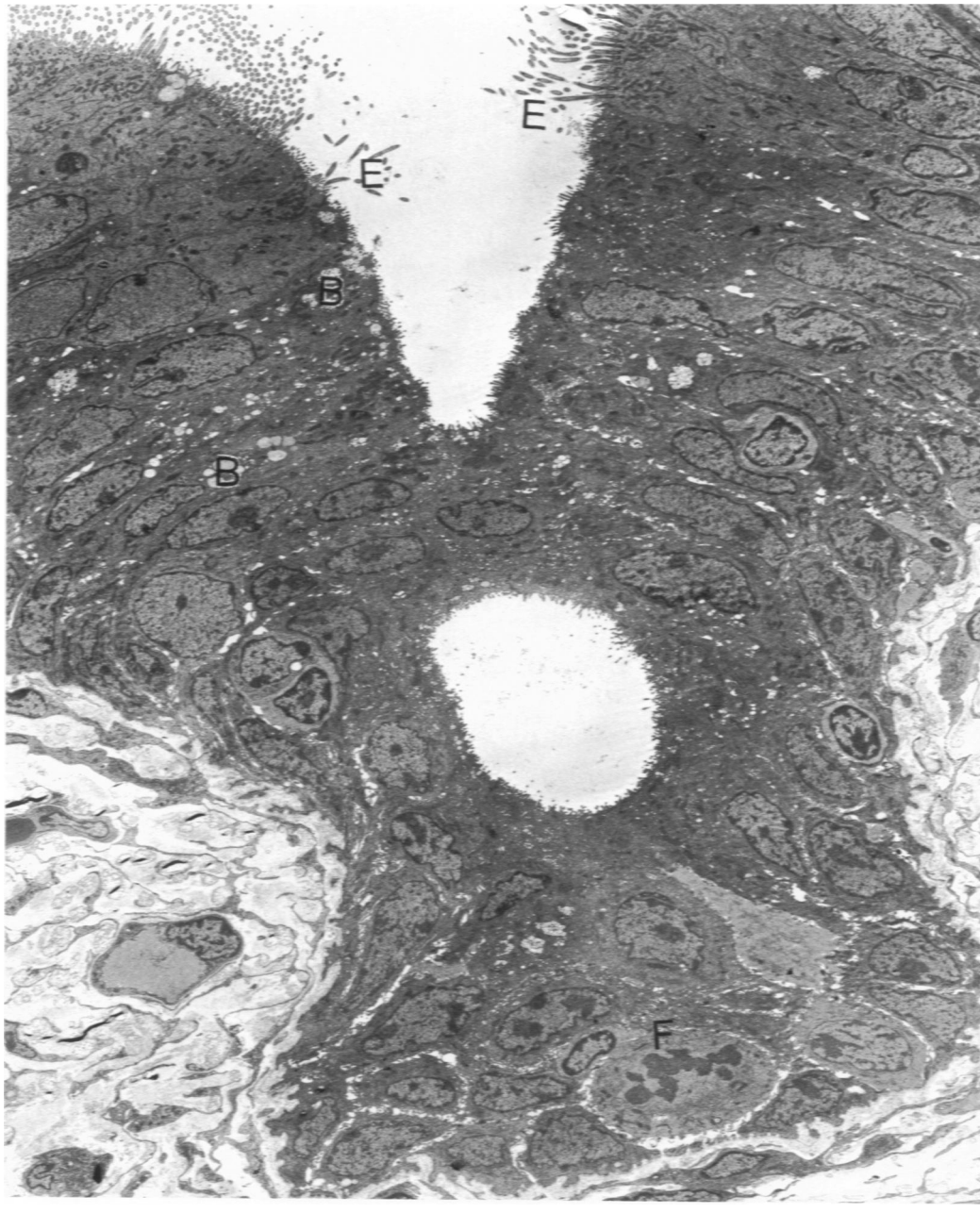
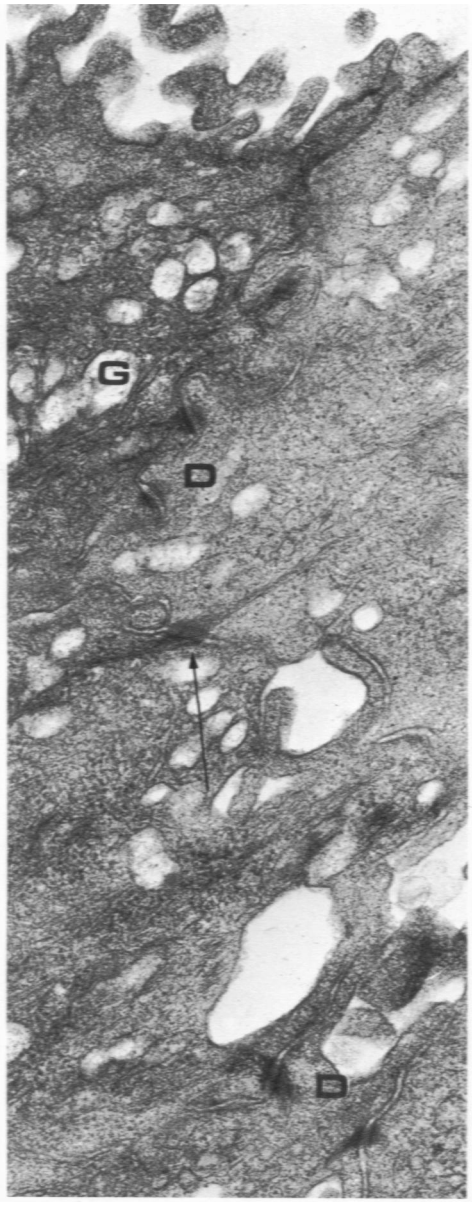


Fig 6—The intermediate lesion. Ciliated cells abruptly discontinue (*E*) at the opening of the duct and are followed by cells with prominent microvilli and residual mucous granules (*B*). There is no increase in layering of cells at the wall of the duct. A mitotic figure (*F*) is present in the ductal wall (Swine TEM, × 1900).



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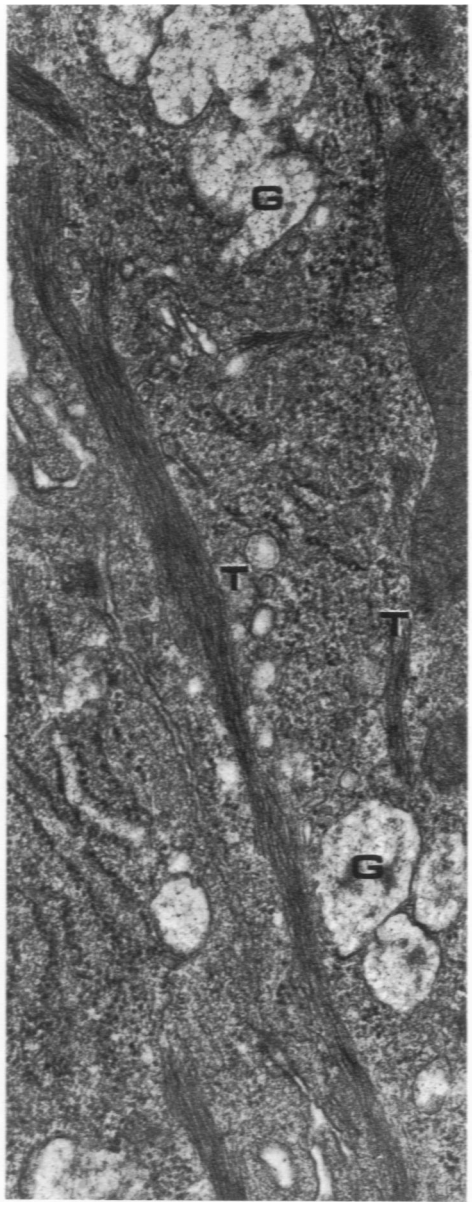

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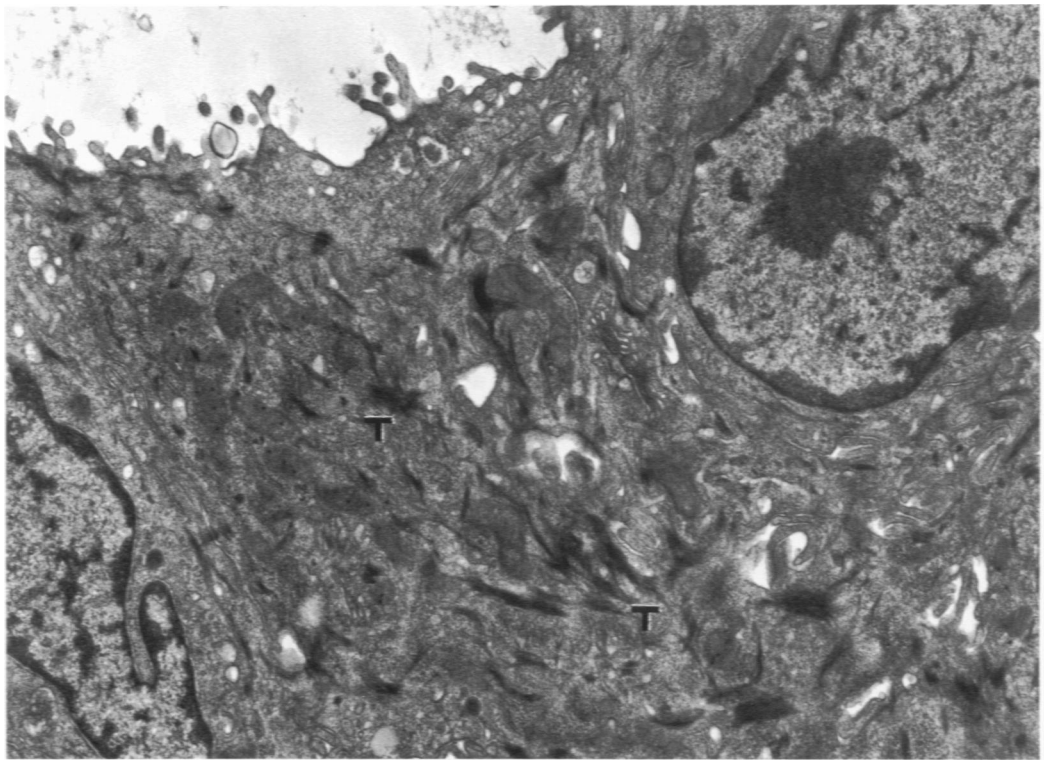
Fig 7, 8 and 9—The intermediate stage of squamous metaplasia. Portions of cells are shown which have varying numbers of mucous granules (*G*) and varying accumulation of tonofilaments (*T*). The number of desmosomes (*D*) between the cells is increased and some are associated with tonofilaments (*arrow*) (Swine TEM, **7,** × 40,000; **8,** × 38,500; **9,** × 16,700). **Fig 10**—Another type of cell with plump microvilli and abundant tonofilaments in cytoplasm is seen between ciliated and goblet cells. Instead of residual mucous granules, small vacuoles (*V*) are found in the cytoplasm (Swine TEM, × 17,400).



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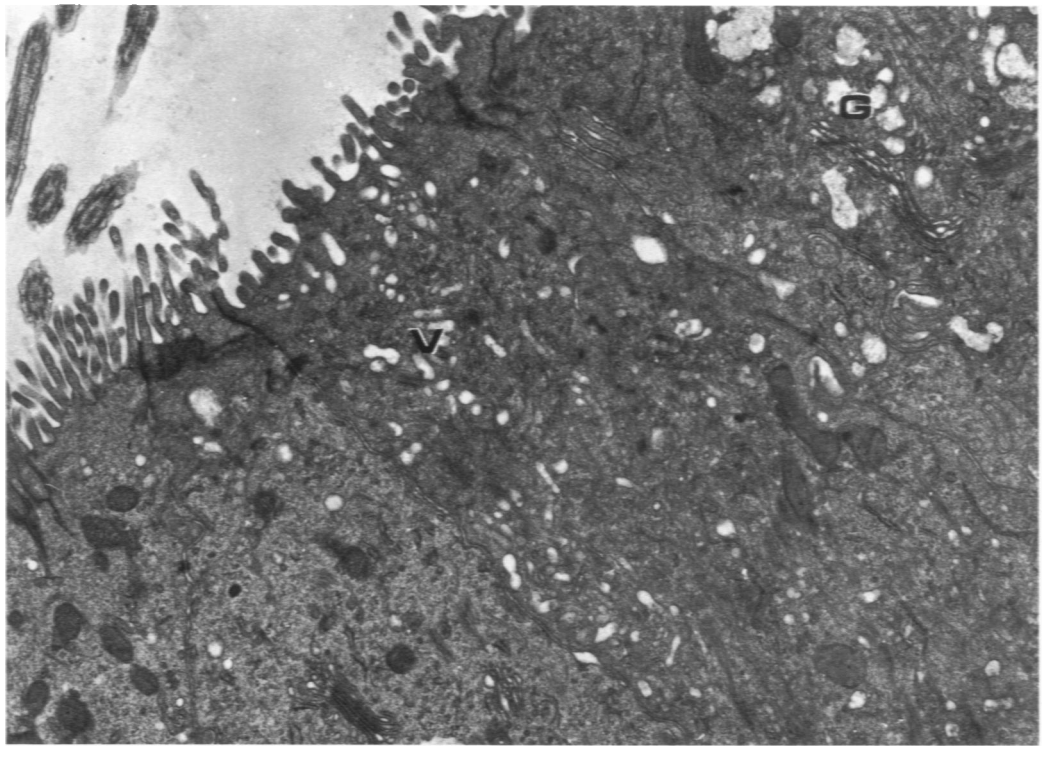

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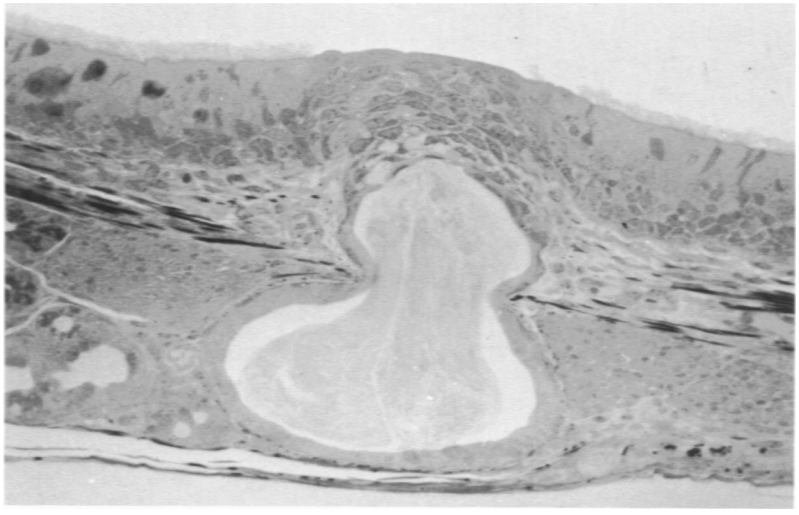

Fig 11 — The advanced stage of squamous metaplasia. Metaplasia is well defined at the opening of the duct. There is increased layering of the cells which accounts for the bulging seen in Figure 4. Mucous plugging and dilatation of the duct are also prominent (Swine TBS, × 350).

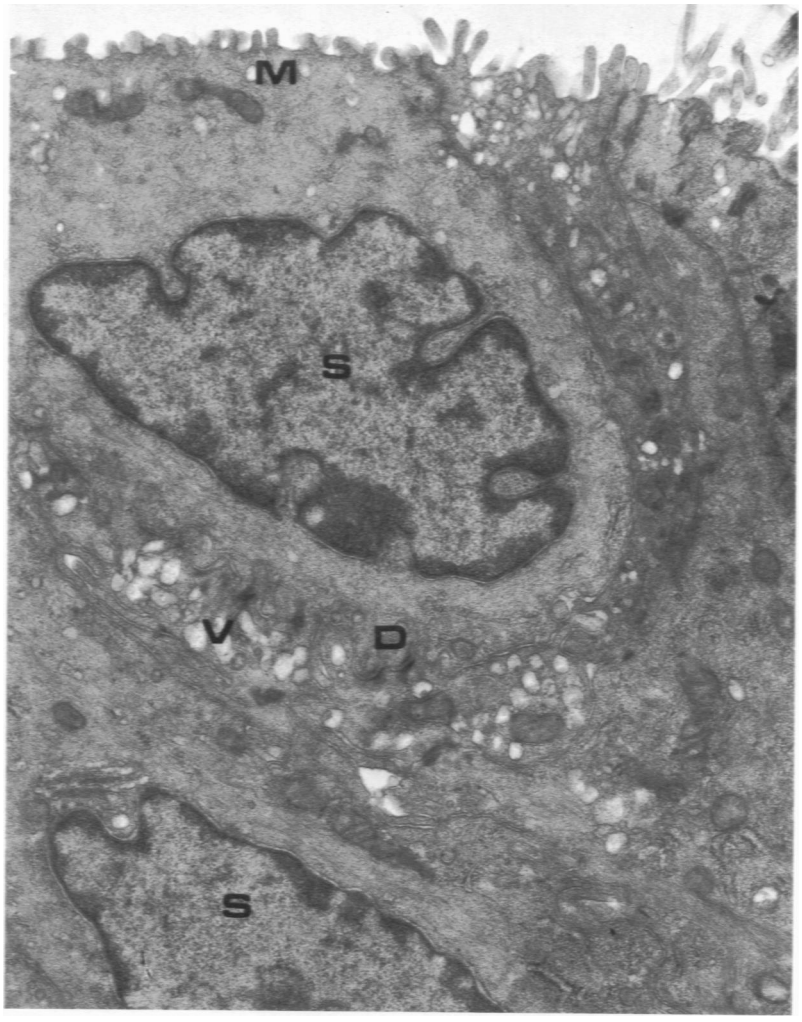

Fig 12 — Noncornified squamous cells (*S*) bordering a ciliated cell from Figure 11 are shown with fine tonofilaments diffusely occupying the cytoplasm. Desmosomes (*D*) are increased, microvilli (*M*) are plump and the nuclei are notched. A cell with abundant small vacuoles (*V*) is also seen (Swine TEM, × 21,000).