ORIGINAL REPORT

SCANNING ELECTRON MICROSCOPY OF THE BRONCHUS AND ADNEXES OF RATS

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ABSTRACT

The bronchial surface and adnexal tissues of rats were observed with the scanning electron microscope. Two kinds of surface cells were identified. Long filamentous cilia covered the free surface of the ciliated cells and frequently extended over the hollows formed by non-ciliated cell surfaces.

Many well-preserved erythrocytes were seen on the somewhat rough surface of the pulmonary vein. The three dimensional features of collagen fibers in subbronchial connective tissue were also demonstrated.

INTRODUCTION

The recent development of the scannning electron microscope has made it possible to demonstrate the stereo-ultrastructure of cell surfaces in detail. This microscope differs from the conventional transmission electron microscope in that the electron beam, instead of passing through an ultrathin specimen, scans the surface of an opaque specimen. The scattered electrons together with secondary electrons emitted by the specimen itself, are then amplified and form an image of the surface on the face of a cathode-ray tube (5). This technique has been used to examine the complex surfaces of dental enamel (3), cornea (2), blood cells (4), sectioned tissue (7), cilia of tracheal and olfactory epithelium (1), synovial membranes (6) and pollen (5).

This paper describes the surface of the normal rat bronchus and adnexal tissues as observed by scanning electron microscopy.

MATERIAL AND METHODS

Normal adult Wistar rats weighing 200-250 g were used. Approximately 1.5 ml of

3% glutaraldehyde, buffered with 0.05 M cacodylate to pH 7.2, was injected into the cervical trachea under light ether anesthesia, and the lungs and trachea were immediately removed. Small slices of the right lung with the right primary bronchus and pulmonary vein were fixed for three hours in the glutaraldehyde solution, washed overnight in Tris-maleate buffer (pH 7.2) which contained 8% sucrose, dehydrated in graded thanol and dried in air. Some shrinkage occurred during air drying.

Small slices, 1×0.5 cm, were coated with evaporated gold in a vacuum-evaporator. They were rotated around the vertical axis while evaporation was carried out at an angle of 45° to the specimen. A JSM-2 scanning electron microscope was used. The accelerating voltage was 25 KV and the beam current was 10^{-11} amp..

RESULTS AND DISCUSSION

Bronchial surface: The conventional transmission electron microscope has demonstrated that the bronchial surface is composed of two kinds of cells ; ciliated cells and non-ciliated mucus secreting cells (8, 9). The former have many cilia with long extensions from the free surface which remove foreign particles from the bronchus and trachea.

In the present observations, the bronchial surface had a number of regularly arranged hollows 10-20 μ apart (Fig. 1), and erythrocytes and a mucus-like substance were frequently seen on the free surface (Fig. 2). The blood cells must become attached there at autopsy.

Higher magnification showed that the free surface was composed of long filamentous structures usually going in the same direction, i.e., cilia. These frequently twined themselves around foreign particles (Fig. 3), and extended over the surface of the hollows (Fig. 4). Where the surface of the hollows was free from cilia it was rather smooth (Fig. 5) and occasionally covered with a mucus-like substance (Figs. 4 and 5). The hollows were considered to be the surface of non-ciliated cells.

The upper bronchus abutting on the bifurcation had somewhat different features. Generally the number of hollows corresponding to the non-ciliated cell surface seemed to be decreased, and the cilia became shorter and thicker (Fig. 5). Barber and Boyde(1) reported that the olfactory and tracheal epithelial cilia of different mammals differ in number, length and internal structure. The cilia of the rabbit trachea do indeed seem to be relatively short and scant and, therefore, they appear more uprighting position in scanning electron microscopy. In the rat bronchus, the long cilia are probably flattened during fixation and dehydration and must have a more upright position *in vivo*.

Venous surface: The surface of the pulmonary vein differed from the bronchial surface and was somewhat rough and contained many blood cells (Figs. 7 and 8). The majority of erythrocytes had a relatively uniform and smooth surface, as shown by Clarke and Salsbury (4) (Figs. 8 and 9). Lymphocytes and other leucocytes were not identified.

Connective tissue: Collagen fibers were also observed in the subbronchial loose connective tissue. Single fibers or bundles of fibers were stretched between both walls across wide spaces, possibly formed during the drying process (Figs. 10 and 11).

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REFERENCES

1) Barber, V.C. and Boyde, A.: Scanning electron microscopic studies of cilia. Zeitschrift für Zellforschung, **84** : 269-284, 1968.
2) Blümcke, S. and Morgenroth, K., Jr.: The stereo ultrastructure of the external and internal surface of the cornea, J. Ultrastruct. Res., **18** : 502-518. 1967.
3) Boyde, A. and Stewart, D.G.: Scanning electron microscopy of the surface of developing mammalian dental enamel, Nature (London), **198**: 1102-1103, 1963.
4) Clarke, J.A. and Salsbury, A.J.: Surface ultramicroscopy of human blood cells, Ibid, **215**: 402 -404, 1967.
5) Echlin, P.: Pollen., Scientific American, **218** : 80-90, 1968.
6) Fujita, T., Inoue, H. and Kodama, T.: Scanning electron microscopy of the normal and rheumatoid synovial membranes., Arch. histol. jap., **29**: 511-522, 1968.
7) McDonald, L.W., Pease, R.F.W. and Hayes, T.L.: Scanning electron microscopy of sectioned tissue, Lab. Invest., **16**: 532-538, 1967.
8) Porter, K.R. and Bonneville, M.A.: An Introduction to the Fine Structure of Cells and Tissues. Lea & Fediger, Philadelphia, 1963.
9) Rhodin, J.A.G. : An Atlas of Ultrastructure, W. B. Saunders Company, Philadelphia and London, 1963.

Explanation of figures

All photographs were enlarged 5 times at printing ; the original magnification is indicated in each figure.

Fig. 1. The bronchial surface is not smooth but has many regularly arranged hollows. ×300
Fig. 2. Erythrocytes and mucus-like substance are observed on the surface. Higher magnification of part of Figure 1. ×1,000
Fig. 3. Higher magnification shows filamentous cilia and mucus-like substance. A few cilia are entwined around foreign particles (left). ×10,000
Fig. 4. Long cilia extended over the hollow surface. A hollow in the center has mucus and a microvillus like structure. ×10,000
Fig. 5. Surface of the upper bronchus abutting on the trachea. The cilia of a cell (center) are not flattened but rather elevated. A non-ciliated cell is also shown (upper left). ×10,000
Fig. 6. A curious structure observed on the upper bronchus, perhaps part of a lymph apparatus. ×300
Fig. 7. A number of blood cells in the pulmonary vein. Most of them are erythrocytes. Lymphocytes and other leucocytes are not identified. ×1,000
Fig. 8. A detail of the vein is shown. An erythrocyte can be seen. ×3,000
Fig. 9. Higher magnification of Figure 7 shows an erythrocyte. ×10,000
Fig.10. Subbronchial space. A section of the bronchus is observed on the right. Collagen fibers are seen in the space. ×100
Fig.11. Higher magnification of a part of Figure 10. A possibly fibrocyte stretches fine fibers in both directions (↑). ×1,000