Cultivation in vitro of Ciliated Epithelium from Human Nasopharynx[1]

Histological distribution of ciliated epithelium in association with other cell types in human nasopharynx has been described by various authors[2,3], while histogenesis has been studied by KANAGASUNTHERAM and RAMSBOTHAM[4]. However, there have been a few attempts to study the ciliated epithelium in tissue culture[5,6]. The present study is part of the work in progress on long and short term cultures of nasopharyngeal mucosa and deals with observations on ciliated epithelium in vitro.

Material and methods. Biopsy material obtained under local anaesthesia through anterior nares by Luc's forceps was used. The material was collected in sterile Gey's balanced salt solution (BSS-G) and cleaned under a dissecting microscope. It was then transferred to BSS-G containing penicillin 100 IU/ml and streptomycin 50 µg/ml, and fragmented. 4–6 fragments were then mounted on surface of each glass culture tubes as well as plastic culture flasks (Falcon) prepared earlier, containing an aliquot 1 ml of a solution of 30% BSS-G, 50% Waymouth's 725/1 chemically defined culture medium and 20% foetal calf serum (2.5 ml for plastic flasks). The fragments were allowed to adhere by incubating the flasks and tubes vertically for 30 min and were then incubated at 37 °C in a roller drum, while plastic flasks were cultured in a stationary state. Cultures were attended to daily and photographs were taken as required. After 6 weeks fine carbon particles were introduced in one of the stationary tubes to study the reaction to foreign material. Histological sections were also prepared whenever feasible.

Results. Cell types. New cells started to grow from the explants after about 2 days. The growth in stationary culture on plastic surface was more uniform, and vigorous than in rotating cultures. This newly formed epithelial sheet (Figure 1) consisted of the following different cell types. Undifferentiated polyhedral and bipolar fibroblast-like cells were present, and in addition patches of ciliated cells with cilia in active motion were seen all over the growing sheets of epithelium. Similarly patches of mucus secreting cells, with cells in different stages of mucus formation from fine mucinogen granules to coarse droplets in process of extrusion, were also seen. Some free droplets moved vigorously in currents of fluid produced by the activity of the cilia from adjacent cells. Other mucus droplets coalesced with each other and the cell debris as well as with carbon particles which were introduced into the culture medium thus forming sticky masses, which increased in size and were kept effectively away from the epithelial surface by active ciliary movement.

Growth pattern and behaviour. Primary growth from the explants as observed previously, was in the form of epithelial sheets. On the 16th day active margin of the fragment showed a second wave of proliferation in the form of buds which formed thickened cellular processes with active cellular tips. These either spread out like a fan or bent and fused with the tips of adjacent processes enclosing a lumen lined by stratified ciliated epithelium (Figures 2 and 3). Quite frequently, a fragment of growing bud was detached and was transformed into a freely floating vesicle lined on the outside with epithelium containing patches of ciliated and goblet cells (Figure 4).

Discussion. This is the first attempt to study the behaviour and growth pattern of ciliated epithelium of human nasopharynx which has been cultivated and studied for more than 16 weeks. The present model system is therefore more promising than short term cultures for different studies of nasopharyngeal epithelium of human nasopharynx. The present study reveals that the epithelial sheet formed in culture had a close resemblance morphologically and functionally to normal epithelium in vivo. Thus ciliated cells propagated in culture continued to maintain their ciliary activity. Similarly, mucous cells continued to produce and secrete mucus. Moreover, the distribution of cells in culture was such that when an epithelial sheet was formed, it was actively able to ward off foreign material and cleanse itself by co-ordinated ciliary movement and mucus production. These functions of individual cells, as well as the cleansing function of the sheet as a whole were observed right from the time of implantation up to this record (16 weeks). The movements of cilia were isochronal[7]. Hence, movements of fluid currents were haphazard and not in any particular direction. Moreover, the active repulsion of

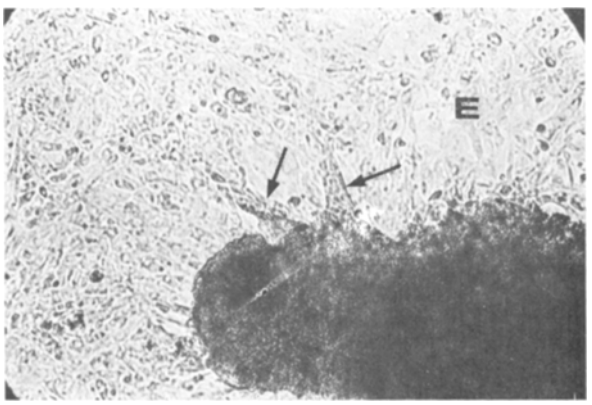

Fig. 1. Live culture showing epithelium sheet (E) and columns (→). × 70.

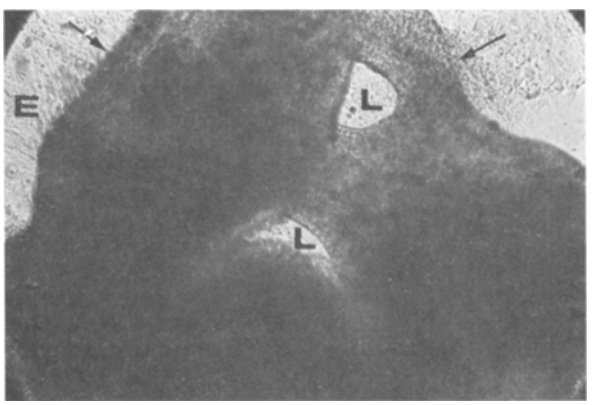

Fig. 2. Live culture showing formation of lumina (L). × 70.

[1] This work, under progress, at the University of Singapore is part of the Ph.D. thesis.
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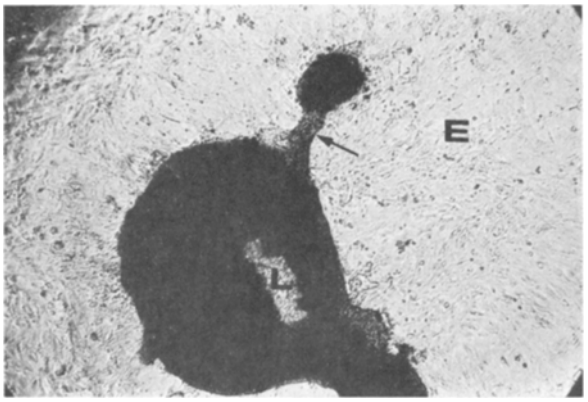

Fig. 3. Live culture showing lumen (L) and separating cyst attached to the cellular process. × 37.

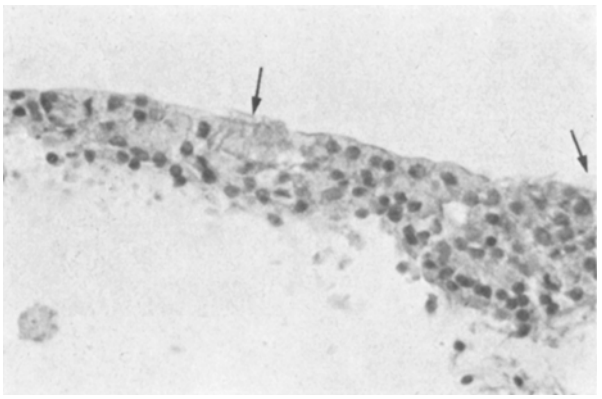

Fig. 4. Magnified view of the lining of the vesicle showing patches of ciliated epithelium (→). × 340.

carbon particles and its binding into mucus droplets were of significance. If formed in vivo, these would go down the pharynx by gravitation. The isochronal ciliary movements as observed in culture could not possibly help the flow in particular direction, but would help the formation of bigger droplets, which would tend to trap the dust, bacteria, and other particles and which could finally be removed by the action of pharyngeal muscles. Thus the experimental studies seem to contradict the usual belief that the ciliary movement occurs in a particular direction. The study of the growth in culture suggests some interesting postulations. The migratory movements (observed from 3rd week onwards) of growing epithelial column which surround foreign materials and cell debris, and the formation of lumina lined with stratified ciliated columnar epithelium as well as its final separation as a cyst, suggest possibly a way of getting rid of more sticky material which cannot be dealt either by mucus adhesion and ciliary movement alone[8].

Résumé. Des cultures d'épithelium naso-pharyngien ont donné naissance à des couches et des colonnes semblables à celles de l'épithelium in vivo. Cette distribution irrégulière de cellules cilifères, de mucosités, et de cellules polyhédriques ont une action coordonnée qui crée un épithelium auto-détergent qui expulse les corps étrangers.

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[8] Acknowledgement. The author is grateful to Prof. R. KANAGASUNTHERAM for his supervision of experimental work and advice in preparing the manuscript, and to Prof. LIM KOK ANN, Professor of Bacteriology, University of Singapore, for providing laboratory facilities. The technical assistance in microphotography of Mr. CHUA BENG CHENG and Mr. J. J. PAREIRA, and histological work of Mr. LEE CHUAN TEE is also acknowledged.

Ultrastruktur der Symbionten in Ovozyten von *Ornithodorus moubata*, Murray (Ixodoidea: Argasidae) nach simultaner Glutaraldehyd-Osmiumfixierung und Nachbehandlung mit Uranylacetat (Triple-Fixation)[1,2]

Mit einer Mischung von Glutaraldehyd und Osmiumtetroxid (OsO_4) konnten Nachteile gebräuchlicher Fixationen wie OsO_4 allein (O) oder Glutaraldehyd gefolgt von OsO_4 (G/O) bei Nierengewebe vermieden werden[3]. Durch zusätzliche Nachbehandlung mit wässriger Uranylacetatlösung wurden Strukturerhaltung und Membrandarstellung von Leukozyten verbessert[4]. Diese leicht modifizierte Triple-Fixation (TF) erzielte ebenfalls gute Resultate an freien Zellen, die mittels Membranfiltration aus kleinsten Volumina von Körperflüssigkeiten gewonnen wurden[5].

In der vorliegenden Arbeit soll der Einfluss der TF auf die Ultrastruktur der rickettsienähnlichen Mikroorganismen gezeigt werden, welche intrazellulär in Zekkenorganen vorkommen[6–13]. Dabei gilt unser Interesse hauptsächlich der Darstellung membranöser Strukturen und der Wirkung des Uranylacetats auf die Verteilung der DNA-haltigen Fibrillen[14,15] und auf die ribosomenartigen Partikel dieser Symbionten.

Material und Arbeitsmethoden. Die hier verwendeten *Ornithodorus*-Zecken stammen aus der Ulanga-Region (Tansania) und werden seit mehreren Jahren am Tropeninstitut gezüchtet.

Die Sektion der Ovarien[16] erfolgte in physiologischer Kochsalzlösung oder direkt im Fixierungsgemisch bei 0–2 °C.

Für die Fixation hielt ich mich, mit geringen Abweichungen, an das Rezept von HIRSCH und FEDORKO[4]. Weil die Ovozyten einen bedeutend grösseren Durchmesser aufweisen als Leukozyten und vor allem in den späten Vitellogenesephasen schwer durchdringbar sind, wurde die Fixierungszeit auf 2 Stunden erhöht.

Fixierungsgemisch (nicht haltbar): 1 Teil A + 2 Teile B (vor Gebrauch bei 0 °C frisch ansetzen).

Lösung A (haltbar): 2,5% Glutaraldehyd in 0,1 *M* Cacodylatpuffer (CP); pH 7,4.

Lösung B (haltbar): 1% OsO_4 in 0,1 *M* CP; pH 7,4.