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التخليق الجنيني لأهداب خلايا الغشاء المخاطي التنفسي للتجويف الأنفي
في الجمل كما ظهرت بالميكروسكوب الالكتروني

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بدأ الغشاء المخاطي التنفسي في النمو على هيئة خلايا غير مهدبة عمودية متعددة الطبقات كاذبة عندما بلغ طول الأجنة ٥٠ مم . وكانت النهايات الطليقة لمعظم الخلايا الطويلة منتفخة وناعمة بينما كان بعضها يحمل عدد قليل من الحليمات الدقيقة القصيرة . بدأت بعض الخلايا في فقد الجزء العلوي المنتفخ تدريجيا عندما بلغ طول الأجنة ٦٠-٨٠ مم ثم يبدأ ظهور بعض الحليمات الرقيقة ، تبدأ الأهداب في الظهور بين الحليمات الدقيقة كان عدد الخلايا المهدبة به مساوي تقريبا لعدد الخلايا الغير مهدبة به في الأجنة التي بلغ طولها ١٤٠ مم ثم فاق كم الخلايا المهدبة به مثيله في الخلايا الغير مهدبة بتقدم الحمل .

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**CILIOGENESIS IN THE RESPIRATORY MUCOSA
OF THE NASAL CAVITY OF THE CAMEL AS REVEALED
BY ELECTRON MICROSCOPY**
(With One Table & 12 Figs.)

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SUMMARY

The respiratory epithelium of the nasal cavity started its development as non-ciliated pseudostratified columnar in foetuses of 50 mm. CVRL. The tall cells had swollen apical portions and their free surfaces were either smooth or carrying scattered short microvilli. At 60-80 mm. CVRL some cells lost gradually their swollen portions, and long, narrow microvilli appeared on their free surfaces. During this stage many basal corpuscles appeared at the supranuclear regions, then arranged themselves under the free cell border and finally young cilia appeared among the latter microvilli and increased in length and density with advancing age. At 140 mm. CVRL, the number of the ciliated cells became nearly equal to the non-ciliated ones, then the former type of cells exceeded the latter during the following foetal ages.

INTRODUCTION

Because of their important functional significance, the surfaces of the respiratory passages of camel have been extensively studied by light microscopy (GEORGE, 1951; TAYEB, 1964 and BADAWI and FATEH EL-BAB, 1974). These surfaces were also studied in other animals using scanning electron microscopy (SEM) which has a unique ability in revealing the size, shape and density of the surface microprojections characterizing the various respiratory cell types.

There was a lack of complete informations from the available literature on the development of the cilia in the respiratory epithelium using SEM. Therefore, the present study focused on the sequence of events of ciliogenesis in respiratory mucosa of camel foetuses using SEM, supplemented with transmission electron microscopy (TEM) in order to correlate the intracellular structure with the surface morphology.

MATERIAL and METHODS

The materials of this study were collected from 12 camel foetuses ranging between 50 and 1000 mm. CVRL (Table 1). The samples were taken from the respiratory mucosa of the nasal cavity at the middle portion of the dorsal nasal concha and immediately immersed in chilled fixative (2% formaldehyde, 1.25% glutaraldehyde in 0.1M sodium cacodylate, pH 7.2) and stored at 4°C for both SEM and TEM.

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Scanning electron microscopy:

The fixed samples were washed in 0.1M sodium cacodylate containing 5% sucrose, processed through tannic acid, dehydrated in graded ethanol series (50%, 70%, 80%, 90%, 95% and 100%) for 15 minutes in each, critical point dried from carbon dioxide, attached to studs with colloidal carbon, and coated with gold palladium in a sputtering device. Specimens were examined and photographed using a SEM operating at 20 KV.

Transmission electron microscopy:

The fixed samples were washed in 0.1M sodium cacodylate containing 5% sucrose and post-fixed in 1% osmium tetroxide in buffer. The tissue was processed through tannic acid, dehydrated in graded ethanol series and embedded in epon. Sections were cut with a glass knife and stained with uranyl acetate for 20 minutes followed by lead citrate for 5 minutes (VENABLE and COGGESHALL, 1965).

Table (1)
List of foetuses used, showing CVRL lengths in millimeters

No. of animals used	CVRL mm	No. of animals used	CVRL mm
1	50	1	200
1	60	1	230
1	80	1	270
1	100	1	450
1	140	1	800
1	170	1	1000

RESULTS

At the early stage of foetal life (50 mm CVRL), the respiratory epithelium of the nasal cavity was of the pseudostratified columnar non-ciliated variety. Most of the cells had swollen apical portions located near each other. The free surfaces of these portions were either smooth or carried few scattered short microvilli. In foetuses of 60-80 mm. CVRL, some cells lost gradually their swollen apical portions through the release of their secretions and numerous, long, narrow microvilli appeared regularly distributed on their free surfaces (Fig. 1). Among these microvilli, young cilia appeared. At this stage the free cell borders were slightly swollen, while the cilia were not dense and sometimes had thickened tips.

The TEM examinations at this stage showed that during the release of secretion, many basal corpuscles identical to the centrosome, were observed at the supranuclear regions. Some of these corpuscles were arranged under the free cell surface and connected to cilia (Fig. 2). Tonofilaments were observed among the latter basal corpuscles. From these, fine filaments extended into the microvilli which were found between the cilia, forming their fine cores. Some large secretory vesicles were observed near the free border of these ciliated cells.

With the advancement of age, the ciliated cells showed a gradual increase in number and the non-ciliated ones became more widely separated from each other (Fig. 3). The dome-

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shaped apical portions of the latter cells carried scattered short microvilli. At the foetal age of 140 mm. CVRL, the number of the ciliated cells became nearly equal to that of the non-ciliated cells (Fig. 4). The cilia became more dense, longer and the microvilli were hidden between them (Fig. 5).

The TEM examinations at the latter age showed light ciliated cells in addition to a few dark ones (Fig. 6). The latter cells were smaller in size and had more dense cytoplasm, darker nuclei and in general had more cilia. The non-ciliated cells also showed light and dark cells. However, the dark cells were few in number, narrow in diameter and looked rather compressed between by the neighbouring cells. Their darker nuclei were irregular in shape and pushed towards the free cell surface.

Gradually with further advancement of age a number of the non-ciliated mucous-secreting cells changed through ciliogenesis by losing gradually their protruded apical portions, appearance of long narrow microvilli on the cell surface and basal corpuscles at the supranuclear regions and finally appearance of young cilia in between these microvilli (Fig. 7). These ciliated cells also arose from newly dividing cells.

The cilia increased in density through the appearance of new younger cilia in between, which rapidly became longer until reaching the mature length. At 200-230 mm. CVRL, the free surface of the mucosa showed irregularly distributed, bulged areas indicating developing processes of mucosal glands. These glands showed clear openings at the free surface in fetuses of 270mm. CVRL (Fig. 8).

At the latter age, the majority of the epithelial cells were ciliated (Fig. 9). The cilia were dense and extended above the level of the swollen apical portions of the non-ciliated cells. Many cells were observed undergoing ciliogenesis, while the non-ciliated cells were in general singly distributed. In fetuses of 800-1000 mm. CVRL, the mucosal glands increased in number, causing clear irregularity to the mucosal surface and have relatively wide openings (Figs. 10, 11). The cilia covered most of the surface and became mature (about 5-6 μ m in length) (Fig. 12). The non-ciliated mucous-secreting cells have a relatively sparse population of short, stubby microvilli. Sometimes these cells were found forming a small group but were often interspersed singly among the ciliated cells. Cells under ciliogenesis were also observed at these late fetal ages.

DISCUSSION

The present data show that the events of ciliogenesis at early foetal life starts by preparing the free borders of the cells for cilia by losing gradually their swollen apical portions through the release of secretions, then appearance of dense, long microvilli and the formation of a great number of basal corpuscles identical to the centrioles. These corpuscles migrate and arrange themselves under the free border and finally the cilia arise. DOUGBAG, *et al.* (1985), described similar but incomplete events of ciliogenesis in the development of the tracheal epithelium.

In the present data, the large secretory vesicles which appeared by TEM in the cells at early ciliogenesis, their slightly swollen apical portions and the SEM pictures suggest that the ciliated cells arise at early foetal life from non-ciliated secretory cells and with the advancement of foetal age they arise further from newly divided ciliated cells. SAGUCH (1917) mentioned that the ciliated cells lose their cilia before the mitotic division after which the cilia arise.

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A dense population of surface microvilli were observed before the appearance of the cilia. This comes in accordance with the findings of RHODIN (1966); ANDREWS (1974) and DOUGBAG, *et al.* (1985). ANDREWS (1974) considered them as a type of microvillus cells.

The present data support the opinion of HAYWOOD (1966); SINGH, *et al.* (1973) and DOUGBAG, *et al.* (1985) that the dark cells of both ciliated and non-ciliated cells are ordinary epithelial cells in the way towards the end of their life span and not an intermediate stage in transformation of a ciliated cell to a goblet cell as mentioned earlier by OSADA (1964) in human tracheal epithelium.

In rats, ANDREWS (1974) reported that the goblet cells were found singly or in small groups among the ciliated cells in the respiratory mucosa of the nasal cavity, which agrees with the present data. This contrasts with the observations of KESSEL and KARDON (1979) who stated that ciliated cells form rows which alternate with others of non-ciliated cells.

The foetal respiratory mucosa of the nasal cavity showed some differences from that of the trachea which was described by DOUGBAG, *et al.* (1985). The former one developed earlier and had more mucosal glands causing bulging areas at its free flattened surface. The latter glands were larger and had wider openings at the surface than those of the trachea, meanwhile the tracheal mucosa was characterized by regular transverse mucosal folds.

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LEGEND OF FIGURES

- Fig. (1):** Scanning electron micrograph, showing luminal surface of respiratory epithelium taken from camel foetus of 80 mm. CVRL. Notice: Swollen apical portions of tall cells (s). A cell which losses its protruded apical portion and carries long, narrow microvilli (m). A cell showing young cilia (c).
- Fig. (2):** Ultrathin section through the respiratory epithelium of 100 mm. CVRL, foetus, showing basal corpuscles (b) found at the supranuclear region. Basal corpuscles arranged in a single row, while the apical portion of the cell still exhibiting a small protrusion (arrow). Non-ciliated, protruded cell (n). Cilia (r). 4,500 X.
- Fig. (3):** Scanning electron micrograph, showing luminal surface of the respiratory epithelium (100 mm. CVRL, foetus), showing apical protrusion of non-ciliated cells carrying few microvilli (short and stubby) (m). Ciliated cell (c) and a cell carrying long microvilli at the beginning of ciliogenesis (b).
- Fig. (4):** At 140 mm. CVRL foetus, the ciliated cells (c) are nearly equal in number to that of the non-ciliated cells (n). A cell undergoing ciliogenesis (b).
- Fig. (5):** Side view of respiratory epithelium, showing apical protrusions (a), cilia (c) and microvilli hidden at the base of the cilia (m). Cut surface (t).
- Fig. (6):** Ultrathin section, showing dark cell (d) in between light ciliated cells (c). Basal corpuscles arranged under the free cell border (b). 3,500 X.
- Fig. (7):** Scanning electron micrograph showing young cilis (c) arising between the narrow microvilli. Non-ciliated cell carrying short microvilli (n). Surface indentation (i) indicating the release of secretion.
- Fig. (8):** The surface of the respiratory epithelium, showing bulged areas (a) above the developing mucosal glands. Opening of glandular duct (o).
- Fig. (9):** At 270 mm CVRL foetus, the ciliated cells became more in number than the non-ciliated cells.
- Fig. (10):** The surface of the respiratory epithelium, showing irregularity caused by the mucosal glands (arrow).
- Fig. (11):** Scanning electron micrograph, showing the opening of a mucosal gland (o). Cilia (c).
- Fig. (12):** The cilia appear mature and cover most of the free surface. (1000 mm. CVRL, camel foetus).















