the intercellular spaces contain the electron dense tracer material. Occasionally, the lanthanum fills a gap junction between adjacent keratinocytes (average, 1 of 24 cells). Without lanthanum, the gap junction is difficult to find and is observed at an average of 1 of 37 cells. After 5 days of vitamin A acid treatment, the lanthanum tracer treated normal and tumor keratinocytes have gap junctions with an average of 1 of 21 cells.

Tumors treated with vitamin A acid show an increase in the Golgi complex and the rough-surfaced endoplasmic reticulum after 2 days of vitamin A acid applications. At this time period, gap junctions as visualized by lanthanum impregnation increase in their frequency of appearance (average 1 of 19 cells).

Lanthanum impregnation depicts the gap junction to be pentalaminar. The median line with its row of discontinuous electron densities is replaced by an uninterrupted electron dense line. The width of this line is 40–50 Å. In oblique section, a pattern of subunits is observed as the gap junction lattice (Figure 1). The width of the plasma membrane measures approximately 75 Å. The total thickness of the lanthanum impregnated gap junction is about 195 Å which is observed in the junction without lanthanum.

Examination after 5 days ot vitamin A acid treatment but without lanthanum depict tumor tissue with 1 of 15 cells having gap junctions between them. Examination after the 5th day of the vitamin A acid-treated tumor tissue also treated with lanthanum yield 1 of 8 cells with gap junctions between them. The intercellular contacts now can assume a very long profile and measure 3 to 4 μm in length (Figure 2).

Discussion. The present study, using the lanthanum tracer technique, positively demonstrates the appearance of the gap junction, particularly after vitamin A acid treatment. Without the use of an extracellular tracer, the gap junction is more difficult to observe and one is sometime unsure of its frequency of occurrence.

Gap junctions have been reported in human epidermis[8,9] basal cell cancer[10], wool follicle cells[11], liver[4], and cervical epithelium[12]. An increasing body of evidence has accumulated indicating the gap junction as a low-resistance pathway for cell to cell coupling. The gap junction lattice also seen in this study, has been suggested as an area to facilitate electronic coupling between cells[13]. This coupling demonstrates areas that exist for the exchange of small ions between cells. LOWENSTEIN[14], as well as other investigators, have suggested that the junction may be instrumental in exchanging substances that control cellular growth and differentiation[15,5]. The gap junction is found to increase in frequency of appearance early in mucous metaplasia. As mucus is copiously produced and secreted from the vitamin A acid-treated keratoacanthoma, gap junctions become the predominant cell junction. It is interesting to speculate that if growth and differentiation depend upon metabolic cooperation through mediators produced by other cells, then the mucus producing keratoacanthoma with its numerous gap junctions can probably act as a syncytium.

Summary. In normal rabbit epidermis or in the untreated skin tumor, keratoacanthoma the usual cell junction is the desmosome. Gap junctions are very sparse. The extracellular tracer material, lanthanum nitrate was used to confirm the definite identification and increase of gap junctions in the vitamin A acid-treated keratoacanthoma. Without the use of lanthanum, the gap may not be always apparent in conventional thin sections and can be confused with the zonula occludens.

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Actin in Tracheo-Bronchial Ciliated Epithelial Cells

In the last few years, contractile proteins, similar to actin and myosin found in smooth muscle cells and blood platelets, have been demonstrated in many non-muscular cells[1]. They have been implicated in cell activities such as motility, division, and exocytosis[1,2]. This paper describes the presence of actin in the apical portion of tracheobronchial ciliated epithelial cells.

The trachea of 7 deeply anaesthetized (i.p. Nembutal, 17 mg/100 g) male Wistar rats (200–220 g) was cannulated and, after bilateral pneumothorax, 3.5–4.5 cm^3 of 10% gelatin solution[3] were injected into the trachea at a constant pressure of 10 cm H_2O in order to prevent damage to cilia. After dissection, the trachea and lungs were dipped into liquid nitrogen for 10 min, and then brought back to a temperature of −20 °C. Longitudinal sections of the trachea and main bronchi were cut on a cryostat, and 2 consecutive sections mounted on the same slide; one was treated with a serum containing anti-actin antibodies (AAA)[4], the other with normal human serum. Both sections were then stained with fluorescein-conjugated IgG fraction of goat antiserum to human IgG[4]. Human tracheal and bronchial samples from one autopsy case without pulmonary disease were collected 1 h after death, frozen in a cryostat, sectioned and treated as above.

For electron microscopic studies, the trachea and lungs of 5 male Wistar rats (150–200 g) were fixed by perfusion[5], and those of 4 rats and 1 human lung were fixed by instillation[3]. Tissues were postfixed in OsO_4 and embedded in Epon[3].

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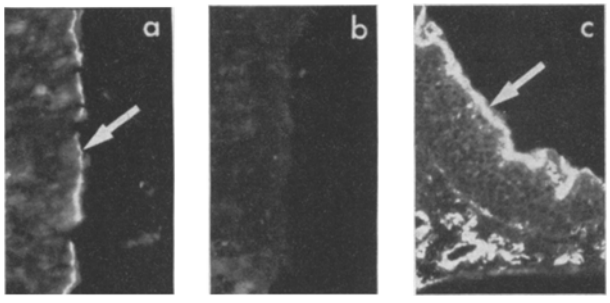

Fig. 1. Immunofluorescent staining of bronchial epithelium with AAA serum. a) Human bronchial epithelium treated with AAA serum shows a sub-ciliary fluorescent band (arrow) ×400; b) this band is absent in a consecutive section treated with normal human serum instead of AAA serum ×400; c) fluorescent pattern (arrow) similar to a) is found in rat tracheal epithelium treated with AAA serum, ×250; note the fluorescent staining of arterial and bronchial smooth muscle.

Cryostat sections of human and rat trachea and bronchi treated with AAA serum showed a sharp fluorescent band parallel to the epithelial surface just beneath the cilia (Figure 1a and c). In control sections, no fluorescence was observed (Figure 1b). After hematoxylin and eosin staining, the AAA-positive subciliar band appeared to be eosinophilic.

Electron microscopic examination of apical portions of ciliated cells indicated that the fluorescent band corresponds to the cytoplasm found between the luminal plasma membrane and the supranuclear mitochondrial accumulation (Figure 2a). This zone, about 0.5 μm deep and devoid of usual cell organelles, contained the ciliary basal bodies with their basal feet and rootlets. Between them, the cytoplasmic matrix appeared dense and granular, containing a mesh of randomly arranged microfilaments (40–80 Å in diameter) which on cross section appeared as fine granules. These microfilaments were frequently in connection with basal feet (Figure 2c), rarely with rootlets.

After incubation with heavy meromyosin[6], the filaments showed the typical arrowhead appearance of actin (Figure 3). Many microtubules were present in the same area (Figure 2b), occasionally closely related to the basal feet (Figure 2c). Some were parallel to the cell surface while others plunged into the dense mitochondrial zone.

Our electron microscopic findings confirm previous descriptions of ciliary structures and of ciliated cells of the lower respiratory epithelium in man and rat[7-10]. Moreover, they show that the dense granular and organelle-free upper zone of ciliated cells contains microfilaments which appear to be a component of the interbasal body apparatus described in nasal respiratory mucosa of the mouse[11]. These microfilaments differ in their structure and location from the tonofilaments binding the basal body to the plasma membrane[12].

The immunofluorescent studies show that actin is located in a regular fluorescent band just beneath the cell surface. The morphology of the microfilaments (40–80 Å in diameter) and their binding with heavy meromyosin indicate that they are composed of actin[1,6,13]. Hence we conclude that the cell matrix between basal bodies contains this contractile protein.

Ciliar movement is currently interpreted as the result of active sliding of the peripheral ciliary microtubules against each other, thanks to dynein arms found on the A subfibre of each pair[14,15]. The presence of actin, related to basal ciliary structures, indicates that, in addi-

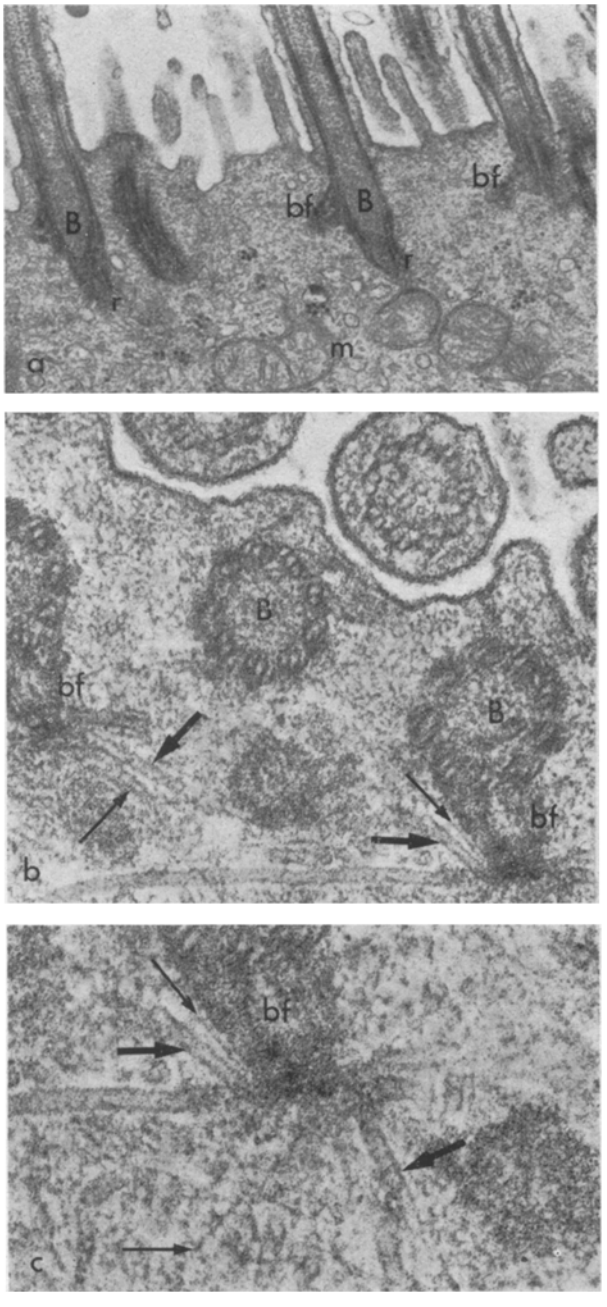

Fig. 2. Electron microscopic appearance of the apical portion of bronchial epithelium. a) Low magnification electron micrograph showing basal bodies (B) in the cytoplasm above the supra-nuclear mitochondrial accumulation (m). Note basal feet (bf) and rootlets (r). ×28,000; b) Microfilaments (thin arrows) and microtubules (thick arrows) in contact with cross-sectioned basal bodies (B) and basal feet (bf). ×86,000; c) Detail from b): microfilaments (thin arrows) and microtubules (thick arrows) are in close relationship with the basal foot (bf). ×124,000.

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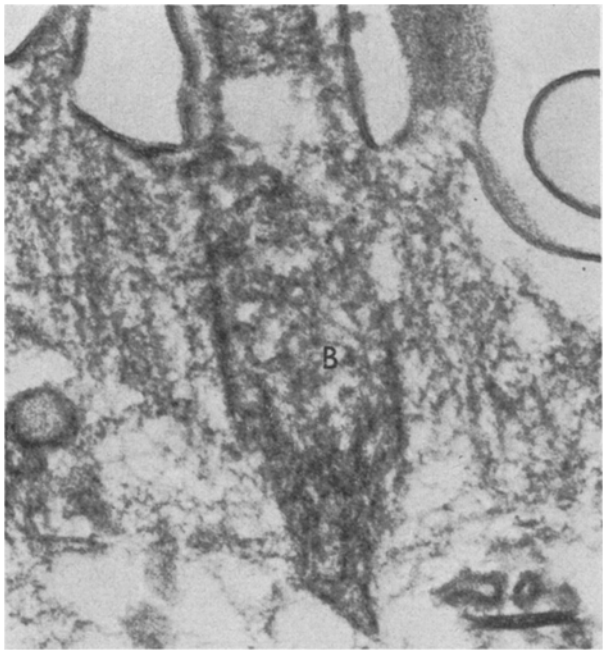

Fig. 3. Electron micrograph of the apical portion of a ciliated cell treated with heavy meromyosin showing arrowhead formations on microfilaments around the basal body (B) (compare with Figure 2). ×110,000.

tion to dynein, other structures may be responsible for the coordination of ciliary movement. The morphology of the basal body, the opposition between basal foot and rootlet, the relation between microfilaments and these structures are highly suggestive of an actively mobile system at the ciliary base. Reinforcing this hypothesis is the fact that cilia beat in a plane perpendicular to that of the central microtubule pair[16], i.e. in the 'basal foot-rootlet' axis. It appears likely that antagonistic forces are implicated in ciliary movement and in its coordination. Actin is an obvious candidate for participating in these phenomena[17].

Summary. The presence of actin has been demonstrated in ciliated cells of the lower respiratory tract by means of immunological techniques and electron microscopy. This protein may play a part in ciliary movement and in its coordination.

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Mise en évidence d'une projection directe des aires hypothalamiques vers le corps genouillé latéral et le cortex visuel chez le chat par la technique de peroxydase

Evidence for Direct Projections from the Hypothalamus to the Lateral Geniculate and Occipital Cortex of the Cat as Demonstrated by Horseradish Peroxidase Technique

Les connexions afférentes et efférentes du corps genouillé latéral (CGL) et du cortex visuel (CxVis) ont été et sont l'objet de nombreuses études anatomiques et physiologiques. Nous avons démontré précédemment[1,2] par la technique de peroxydase ('horseradish peroxidase, HRP') les afférences extrarétiniennes du noyau dorsal du CGL (CGLd) ainsi que du CxVis, provenant de la formation réticulée ponto-mésencéphalique. Le but de cette note est de démontrer que les aires hypothalamiques envoient également des projections directes vers le CGLd et le CxVis, projections dont on n'avait pas, jusqu'alors, de preuve anatomique ni physiologique.

Matériel et méthodes. Chez 12 chats, des volumes allant de 0,1 à 1,0 µl de HRP 33% (Sigma, Type VI) on été injectés dans le CGLd avec une seringue Hamilton de 5 µl. Chez 4 autres chats, une injection de 3 µl de HRP 50% a été effectuée en trois points du gyrus latéral postérieur correspondant à l'aire 17 et à la partie postérieure de l'aire 18. Les temps de survie ont varié de 13 à 72 h. Les techniques employées pour le sacrifice des animaux et la mise en évidence de HRP ont été décrites dans un article précédent[1].

Résultats et discussion. 1. Afférences du CGLd provenant des aires hypothalamiques. Des neurones marqués par HRP sont observés bilatéralement dans des aires hypothalamiques relativement restreintes situées immédiatement sous la surface basale du cerveau (Figure A). Dans le plan le plus rostral (A10), les neurones sont situés latéralement au fornix (Fx) et médialement à la capsule interne (CI), dans une région correspondant en partie au faisceau médian du télencéphale ('medial forebrain bundle, MFB'). Plus caudalement (A9, A8), les neurones apparaissent dans l'aire hypothalamique latérale (HL) et dans le noyau du tuber, latéralement au corps mamillaire et médialement au pédoncule cérébral et au noyau subthalamique. Ainsi la topographie des neurones correspond-elle en partie au tractus optique basal décrit par GILLILAN[3].

2. Afférences du CxVis provenant des aires hypothalamiques et du mésencéphale antéroventral adjacent. a) Projection des aires hypothalamiques vers le CxVis. Dans les plans les plus rostraux (A14, A13, A12), quelques neurones marqués sont observés dans la région préoptique (RPO) et dans le noyau supraoptique (SO). Dans les plans A11 et A10, de nombreux neurones sont situés dans l'aire hypothalamique latérale, le noyau tuberomamillaire et dans le noyau périfornical de CHRIST[4]. Quelques neurones des aires hypothalamiques dorsale et postérieure sont également marqués. Plus caudalement, de nombreux neurones continuent à apparaître dans l'aire hypothalamique latérale et dans le noyau tuberomamillaire. On

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