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A BIPHASIC CHAMBER SYSTEM FOR MAINTAINING POLARITY OF DIFFERENTIATION OF CULTURED RESPIRATORY TRACT EPITHELIAL CELLS

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SUMMARY

A simple, disposable, biphasic cultivation chamber has been developed for respiratory tract epithelial cells. This chamber, the Whitcutt chamber, contains a movable, transparent, permeable gelatin membrane that can be employed either submerged in the culture medium, thereby feeding the cells by the traditional immersion method, or raised to the surface of the culture medium, to bring the apical surfaces of the cells into contact with air and provide nutrients only from below (basal feeding). The effects of biphasic cultivation on the growth and differentiation of respiratory tract epithelial cells from different sources have been studied in Whitcutt chambers. Primary hamster tracheal epithelial (HTE) cells grown to confluence with basal feeding developed a ciliated columnar morphology, with differentiated features (cilia and mucous granules) located in the apical region of the epithelial layer. These cells secreted mucinlike molecules from the apical surface (i.e. the surface in contact with air). Although the apical localization of differentiation features was greater, mucous cell differentiation achieved by basal feeding was quantitatively not greater than that achieved by continuous immersion feeding. Similarly, basal feeding did not alter the degree of epithelial cell differentiation in cultures derived from rat, rabbit, and monkey tracheas or from human bronchial and nasal tissues. In contrast, the differentiation of guinea pig tracheal epithelial cells in culture was significantly influenced by the feeding method employed. When fed basally, guinea pig tracheal epithelial cell cultures expressed various mucociliary functions with resemblance to mucociliary layers in vivo, whereas constantly immersed cultures seemed stratified and squamous. These results suggest that, at least for guinea pigs, the combination of feeding methods provided by the Whitcutt chamber can be used to achieve differentiated cultures of tracheal epithelial cells with a polarity of differentiation that is similar to that observed in intact airways in vivo.

Key words: airways; differentiation; biphasic cultivation; polarity; mucous cells; epithelial cells.

INTRODUCTION

Polarity, or asymmetrical expression of cellular functions, is a normal condition in vivo for many epithelial cell types. In vivo concentration gradients of nutrients and growth-regulating factors are maintained across basement membranes and cell layers. Cells may attach to a basement membrane and feed on the attachment side (the basal surface), while exhibiting specialized differentiated properties at the opposite apical surface.

Alternatively, layers of cells may be progressively more differentiated the further they are situated from the supporting stromal material. Studies in vitro have demonstrated that the substratum can influence dramatically the survival and growth of epithelial cell types (1,2,4,5,8,10,13,16), and can affect their response to specific growth factors and their expression of differentiated functions (2,3,7).

We have previously developed a serum-free hormone-supplemented medium that supports growth and differentiation of tracheal epithelial cells from various rodents (11,13,14), humans (11,12,15), and monkeys (in preparation). When tracheal epithelial cells are cultured in this serum-free medium on collagen gel substrata, the degree of differentiation ranges from limited mucin synthesis to full expression of specialized features of mucociliary epithelium (e.g., mucus-secreting granules and cilia), depending on the species (11). In those studies (11), the cells were immersed in the culture medium, in accordance with traditional cell culture techniques. The

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polarity of differentiation, such as cilia and mucous granules in the apical cell layers, was not well maintained. The morphology of the cells also differed from that observed in vivo.

In an effort to achieve greater differentiation in vitro and, in particular, to achieve the polarity of differentiation seen in vivo, a new system for culturing airway epithelial cells has been developed. This system is based on a specially constructed cultivation chamber, the Whitcutt chamber, which contains a permeable gelatin membrane that can be either submerged in the culture medium, for immersion feeding, or raised to the surface of the culture medium, to bring the cells into contact with air and provide the cells with nutrients only from below (basal feeding). In this report, we describe the construction of the Whitcutt chamber and the results of culturing primary airway epithelial cells from different species in this system.

Materials and Methods

Cell isolation and culture conditions. Syrian hamsters and rabbits, aged 2 to 5 mo. were obtained from Engle Lab (Farmersburg, IN); guinea pigs (male, Hartley strain, 250 to 300 g) and Fischer 344 rats (either sex, 2 to 3 mo.) were obtained from Charles River of Canada (Kingston, Ontario). Human nasal and bronchial tissues were kindly supplied by Drs. J. Yankaskas, M. Knowles, and R. Boucher (University of North Carolina, Chapel Hill, NC). Cells were isolated from these tissues following the established procedures employing protease digestion, as described previously (11-14). The yield for guinea pig trachea was 2 to 4 million cells/trachea; cell yields from the other tissues have been reported previously (11). It should be noted that this isolation procedure results in cultures containing less than 1% fibroblasts (13).

Primary cultures were initiated by plating 1 to 5 $\times$ 10^4 cells/35-mm culture dish or Whitcutt chamber (*see* below for description of Whitcutt chamber). Both containers were coated with 0.6 ml collagen gel (0.24%) (13). The serum-free culture medium for the various epithelial cell cultures consisted of Ham's F12 medium supplemented with insulin (5 μg/ml), transferrin (5 μg/ml), epidermal growth factor (25 ng/ml), hydrocortisone (1 μM), cholera toxin (40 ng/ml), bovine hypothalamus extract (BHE, 15 μg/ml), and vitamin A (retinol, 0.1 μM). BHE was prepared according to the procedure described by Maciag *et al.* (9). Hormonal stocks were prepared at concentrations of 500$\times$ or 1000$\times$, as described previously (11-14). Vitamin A stocks dissolved in dimethyl sulfoxide were stored in liquid nitrogen at a concentration of 10 000$\times$ and were used under yellow lighting to minimize photodegradation. For cultures in both culture dishes and Whitcutt chambers, medium changes were performed 2 d after plating and every 4th d thereafter. Confluent primary cultures were routinely observed within 7 to 10 d.

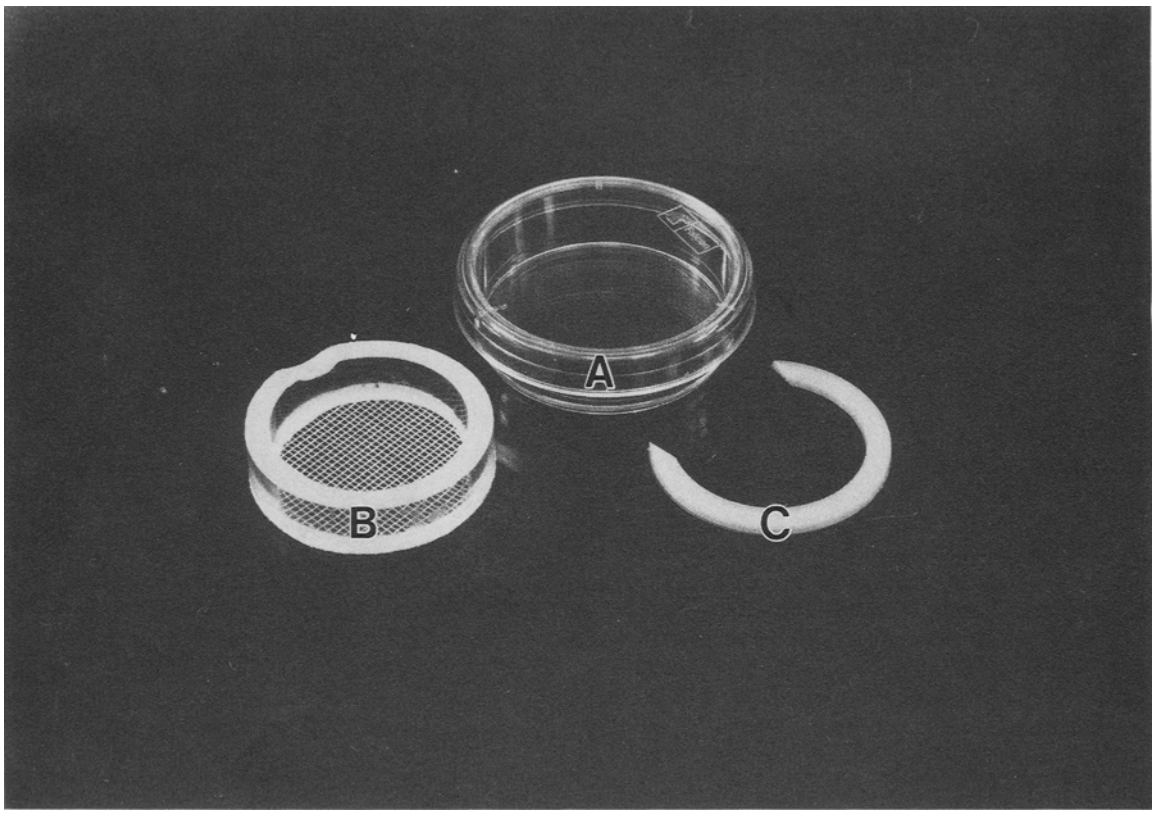


FIG. 1. New biphasic culture system, the Whitcutt chamber, developed for growing respiratory tract epithelial cells between air and liquid phases. *A*, 35-mm culture dish; *B*, chamber with nitrocellulose- or polycarbonate-gelatin membrane; *C*, the support to raise the Whitcutt chamber culture above the culture medium.

Confluent cultures can be passaged 2 to 3 times (11-14); however, only primary cultures were used in this study.

Preparation of the Whitcutt chamber. The Whitcutt chamber consists of three parts (Fig. 1): *A*, a plastic tissue culture dish, 35 mm for single chambers, larger for groups of chambers; *B*, the chamber itself, which consists of a polycarbonate ring (1.25" o.d. × " i.d. × 5/16" high) glued on one side to a 100-nm pore size polycarbonate (Nucleopore) or nitrocellulose membrane cross-linked to gelatin; and *C*, a plastic, autoclavable spacer, 2 to 5 mm thick to raise the chamber when basal feeding is desired. The chamber was prepared by the following protocol. A 2% (wt/vol) solution of gelatin (Difco, Detroit, MI) was prepared at 90° C in a 25% (vol/vol) solution of glycerol in water and stored at 4° C in 25-ml glass bottles (25 ml is sufficient to prepare five membranes). The gelatin solution was melted (by placing the bottle in a beaker of warm water) and poured into a 150-mm glass Petri dish (maintained at 40 to 60° C and kept covered whenever possible). Nitrocellulose or polycarbonate filters, 90 mm in diameter, were floated individually on the gelatin solution (avoiding air bubbles), turned over to wet the other side, and then placed on a 90 mm diameter Whatman acrylic filter support plate attached to a Büchner funnel. To prevent the filters from drying out, it is advantageous to keep a wet filter paper between the acrylic support and the nitrocellulose or polycarbonate filter. The filters were briefly vacuum dried, one at a time, and then were packed between spacer sheets of Whatman no. 54 filter paper (110 mm diameter) in a large beaker. The filters were incubated at 4° C for 1 h and then flooded with cold ethanol (100%). After 1 h, the ethanol was decanted and the filters were rinsed with several changes of cold water over a period of 3 to 18 h. The pack of filters was then fixed in cold 2.5% glutaraldehyde. The glutaraldehyde was decanted after 1 h, and the pack was washed twice with cold water before being suspended in a 0.1% solution of sodium borohydride. After incubation at 4° C for 3 to 18 h, the sodium borohydride was poured off and the pack was rinsed at least three to four times with cold water before being stored in 70% ethanol at 4° C. Preparation of the membranes was completed at room temperature. For each membrane, a single gelatin, cross-linked nitrocellulose or polycarbonate filter was sandwiched between a pair of nylon, 124-mm mesh spacers (Millipore Corp.), and the uncomplexed components of the filter were dissolved by successive 5-min immersions in methanol, acetone (twice), and acetone-ethanol (1:1). The resulting membrane was treated with acetone, methanol, and acetone washes at 5-min intervals to adjust its size and improve its adhesion to the nylon mesh. The membrane was equilibrated with 25% glycerol in methanol for 5 min (to prevent it drying out), then was carefully drained, blotted on absorbent paper, the upper mesh removed, and the membrane and lower mesh allowed to dry in air for 30 min. To construct the chamber unit, a polycarbonate ring was coated with silicone glue, pressed onto a dry membrane, and allowed to set over 2 h. To ensure a watertight seal, the polycarbonate membrane junction was lightly coated with 2% agarose (Sigma, St. Louis, MO, type VII #A-4018) and cooled to 4° C for 5 min. The completed chambers were sterilized overnight in 70% ethanol and rinsed with water before use.

To initiate a primary culture in the Whitcutt chamber, the chamber was placed in its culture dish, the membrane was overlaid with 0.6 ml collagen gel as described previously (13), and dissociated tracheal epithelial cells in serum-free medium were plated on top of this substratum. The chamber was kept immersed in the culture medium for 4 d, then the spacer (Fig. 1 *C*) was inserted below the chamber to raise the chamber to the surface of the culture medium. Care must be taken at this stage to avoid any air bubbles becoming trapped under the chamber. Care should also be taken to avoid overfilling and reimmersing the raised culture during medium changes.

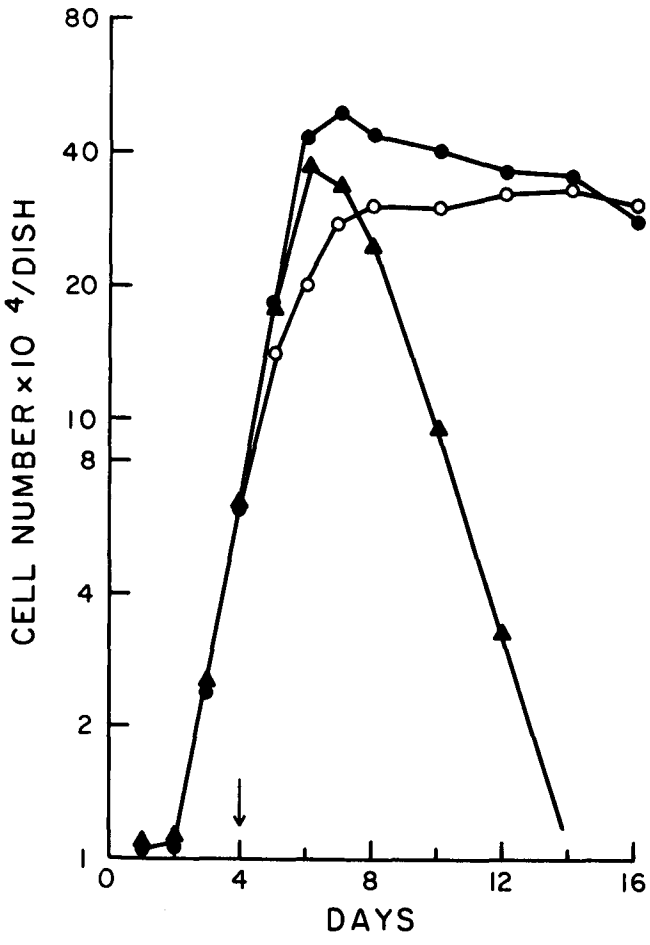


FIG. 2. Growth of primary hamster tracheal epithelial cells in culture. Protease-dissociated hamster tracheal epithelial cells were plated on a tissue culture dish (●) or Whitcutt chamber (○, ▲) at a density of 2 × 10^4 cells/dish. After a 4-d incubation in immersed feeding (*arrow*), some of the Whitcutt chamber cultures were raised for basal feeding (○). A continuous immersion of the Whitcutt chamber culture in the medium (▲) caused cell detachment and reduced the cell number in each dish. Duplicate dishes were used to determine cell number at each time point. The means of these determinations are plotted; the variation in duplicate dishes is 10 to 20% of the mean.

Histochemical and ultrastructural analyses. For histochemical studies, cultured cells were dissociated from the culture dishes or Whitcutt chambers with 0.1% trypsin-5 m*M* EDTA solution, and the dissociated cells were spun on glass slides in a cytospin centrifuge. These slides were fixed in 10% formalin and stained by the periodic acid Schiff (PAS) method. PAS-positive cells were counted as described previously (13). Transmission and scanning electron microscopic studies were performed according to reported procedures (13), using Phillips 300 TEM or Zeiss EM 10C and Amray SEM scopes, respectively.

Biochemical analyses. The radiolabeled glycoprotein precursors: [^{3}H]glucosamine (10 μCi/ml), [^{35}S]sulfate (10 to 50 μCi/ml), and [^{3}H]serine (10 μCi/ml) were used, separately or in combination, to label molecules produced or secreted by the cultured cells. When Whitcutt chambers were used, the contents of three compartmental fractions were examined. For the medium-associated fraction, the cells' culture medium was collected and centrifuged to remove any cell debris. For the lumen-associated fraction, the apical surface of the cultured cells was washed three times in 0.5 ml phosphate buffered saline (PBS)-EDTA (1 m*M*) to collect any secreted products, and the pooled washes were centrifuged to remove any debris. For the cell-associated fraction, the cells and the collagen gel substratum were lysed with 1 ml of sodium dodecyl sulfoxide (SDS) sample buffer containing 5% β-mercaptoethanol (6). These three fractions were then treated with hyaluronidase (10 U/ml) in acetate buffer, pH 6 (12,13), for 24 h at 37° C. The hydrolysates were analyzed by Sepharose CL-6B column chromatography, as described previously (12,13).

Sources of chemicals. All tissue culture media and antibiotics, except Gentamycin sulfate (Schering Co., Kenilworth, NJ), were products of GIBCO (Grand Island, NY). Hormones and growth factors were obtained from Collaborative Research Inc. (Waltham, MA). Trypsin (2× crystallized), protease (type 14), and vitamin A were from Sigma. Sepharose CL-6B was from Pharmacia Fine Chemicals (Piscataway, NJ). Hyaluronidase (Streptomyces) was purchased from Miles Laboratories (Elkhart, IN). Collagen solution (Vitrogen 100™) was obtained from Collagen Co. (Palo Alto, CA). Radiolabeled precursors: [^{3}H]6-glucosamine (30 Ci/mmol), [^{3}H](G)-serine (150 mCi/mmol), and [^{35}S]sulfate (carrier-free) were obtained from ICN (Irvine, CA). The remaining chemicals were all of analytic grade.

RESULTS

Growth of hamster tracheal epithelial cells in Whitcutt chambers. Preliminary studies indicated that primary epithelial cells from various animal tracheas cannot attach and grow in Whitcutt chambers without a collagen gel substratum. Therefore, collagen gel was layered on top of the chamber's membrane for all studies reported here. It is possible that other cells, e.g. HeLa, WI-38, or MDCK cells, would not need this collagen gel substratum to grow in Whitcutt chambers (Whitcutt, J. M., unpublished data).

Hamster tracheal epithelial cells fed by either the immersion or basal feeding method grew well in Whitcutt chambers. Cultures that were continuously immersed in the culture medium, however, exhibited decreased stability and reproducibility compared to cultures on collagen-coated plastic tissue culture dishes or cultures fed basally in Whitcutt chambers (Fig. 2). Examination of the culture medium revealed that the hamster cells produce a substance with collagenaselike activity, which would be able to digest the collagenous substratum (13). This could explain the detached cells that were seen floating in the cultures, especially the late primary cultures, grown in Whitcutt chambers and fed only by the immersion method. Presumably, the firm support provided by the plastic surface of the tissue dish compensated for the potential instability of cultures on collagen-coated tissue culture dishes.

In contrast to the continuously immersed cultures, hamster tracheal epithelial cells that were cultured in the raised position, after 4 d in the immersed position,

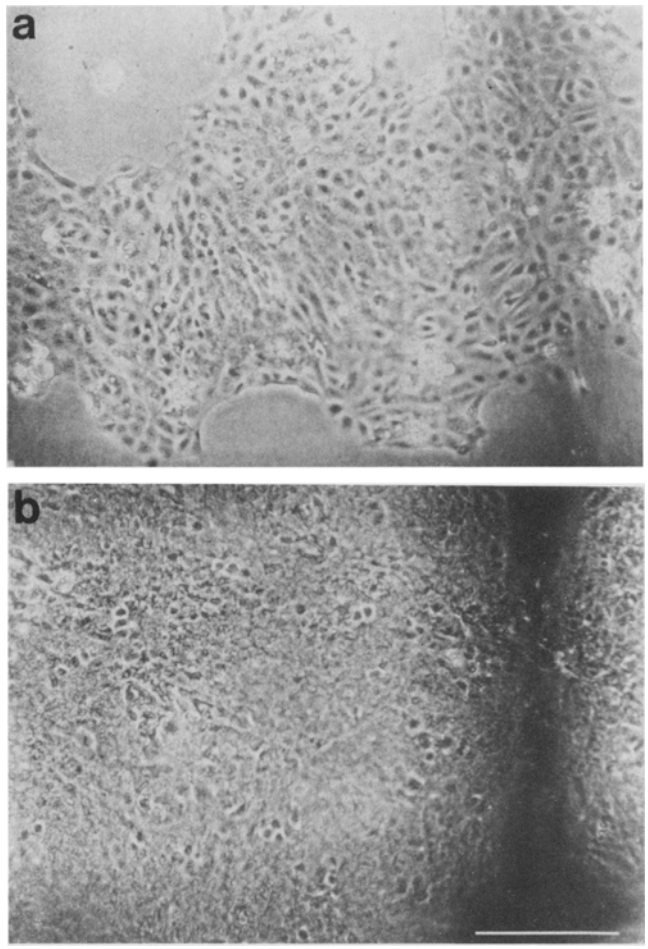


FIG. 3. Phase contrast photomicrographs of hamster tracheal epithelial cells growing in a Whitcutt chamber, demonstrating the excellent optical advantage of this system over several commercial preparations. *A*, 2-d-old culture; *B*, 14-d-old confluent culture. *Bar* = 200 μm.

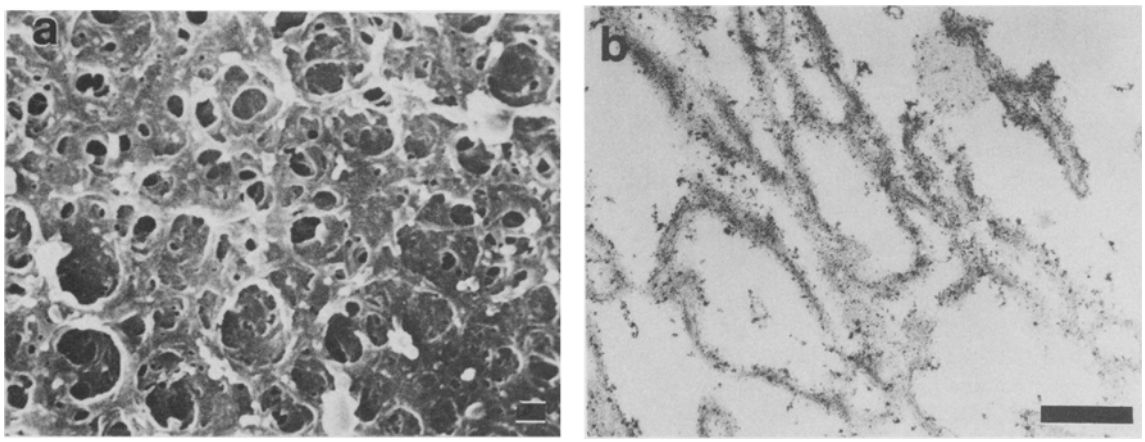


FIG. 4. Electron micrographs of the porous nature of the membrane in the Whitcutt chamber. *a*, scanning electron micrograph; *b*, transmission electron micrograph. *Bars* = 100 nm.

exhibited increased cell number with time, good stability, and good reproducibility (Fig. 2). Thus, cultured hamster tracheal epithelial cells are able to proliferate in this specialized chamber when one side (the apical side) of the cell layer is exposed to air, while nutrients and growth factors are obtained from the opposite side (the basal side) of the cell layer.

The transparency of the membrane in the Whitcutt chamber facilitates examination of living cells in either the immersed or raised position (Fig. 3). This is a clear advantage over some commerically developed chambers (e.g., Millicell TM-HA from Millipore Corp., Bedford, MA), in which the membrane is translucent. Furthermore, the porous nature of the membrane in the Whitcutt chamber (Fig. 4) permits translocation of most nutrients and peptide hormones. Thus, although cells cultured in the raised position are not immersed in the culture medium, and therefore are not in direct contact with several vital growth factors, they seem to be able to receive these substances through the membrane and collagen substratum, i.e. from their basal surfaces, and the cells continuously proliferate when "fed" in this manner.

Differentiation of hamster tracheal epithelial cells in Whitcutt chambers. At 7 to 14 d after the start of basal feeding, the hamster tracheal cells seemed to differentiate into ciliated and mucus-secreting cell types (Figs. 5 and 6). Electron microscopic examination of the cultured cells confirmed the presence of cilia in some of the cells (Fig. 5). The cilia had cytoplasmic basal bodies and the usual 9+2 microtubule doublets pattern in the ciliary axonemes (Fig. 6). Other cells seemed to have mucous granules and presumably mucous cells (Fig. 6). In both cases, the differentiated features occurred in the apical regions of the cultured cells. In contrast, the middle layer cells of the culture were cuboidal and undifferentiated (Fig. 6 *A*). It should be pointed out that the number of cell layers on top of collagen gel substrata is very heterogeneous in culture; it ranged from one to several cell layers (Fig. 6). However, cells in many monolayer areas were morphologically similar to columnar epithelia

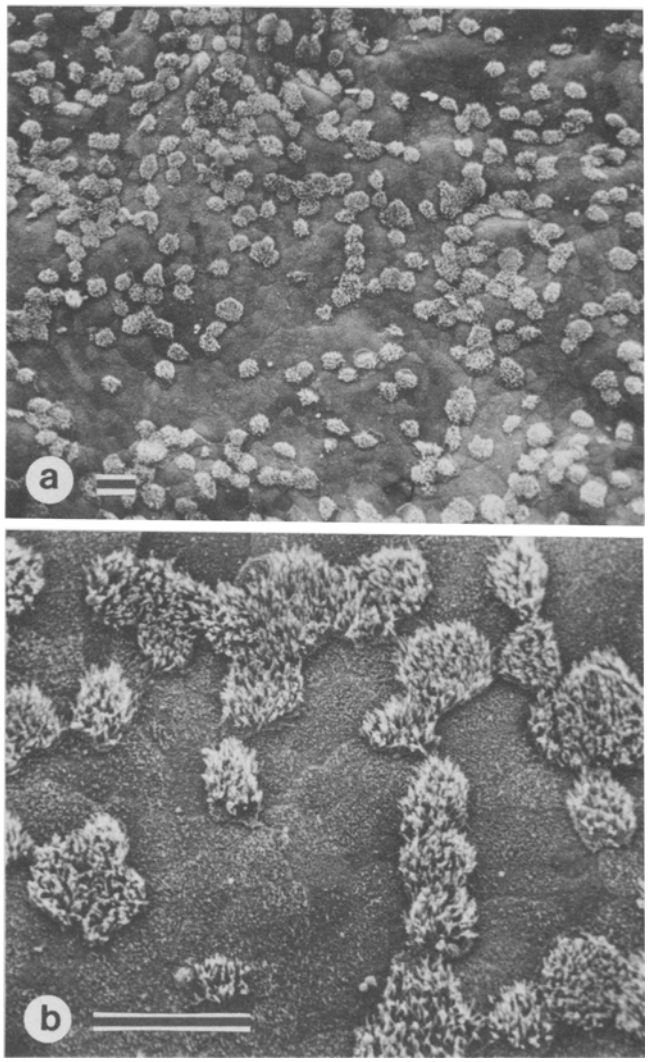


FIG. 5. Scanning electron micrographs of hamster tracheal epithelial (HTE) cells cultured in the Whitcutt chamber for 14 d, *b* is an enlargement of *a*, showing ciliated epithelium. *Bar* = 200 μm.

in vivo (Fig. 6 *B*). This morphologic differentiation is elevated in the biphasic culture condition. A similar phenomenon has not been observed in cultures that were traditionally maintained.

The results of biochemical analyses of the secretory products from these cultured cells support the morphologic indications that mucous cell differentiation occurs in the Whitcutt chamber. Analyses of the products secreted or stored in the three compartmental fractions revealed differential distribution of molecules of various sizes (Fig. 7). Most labeled molecules in the cellular- and lumen-associated fractions were excluded from a Sepharose CL-6B column, indicating the presence of high molecular weight molecules in these two compartments. In contrast, the labeled molecules in the medium-associated fraction were predominantly of low molecular weight.

The properties of the high molecular weight labeled molecules were glycoproteinlike, inasmuch as most of them were labeled with [^{3}H]serine (data not shown), [^{3}H]glucosamine, and [^{35}S]sulfate. Furthermore, 85% of these labeled molecules in the void volume fractions exhibited biochemical properties that are common to mucinlike molecules (13), such as resistance to enzymes that specifically degrade various proteoglycans (e.g., hyaluronidase, chondroitinase, heparinase, heparitinase) and resistance to enzymes that cleave N-type glycoproteins (e.g., *N*-glycanase, endoglycosidases F, and H) (13).

Furthermore, composition analyses of these glycoproteinlike molecules by high performance liquid chromotography revealed that the linked sugar between the protein and carbohydrate chains was *N*-acetyl galactosamine (13).

Quantitative analyses of mucous cell differentiation in culture. To determine whether new differentiation occurs in this new culture system, morphometric and kinetic studies of cell differentiation were performed. Quantitation of mucous cell differentiation was performed by the PAS method, as described previously (13). The levels of PAS-positive granules in the cultures correlated closely with the kinetics of mucin biosynthesis, measured by incorporation of radiolabeled glycoprotein precursors into high molecular weight, hyaluronidase-resistant molecules in the cellular- and lumen-associated fractions (13). Immersed cells in tissue culture dishes and basally fed cells in Whitcutt chambers expressed similar relative frequencies and kinetics of mucous cell differentiation (Table 1).

Effects of basal feeding in Whitcutt chambers on the differentiation of tracheal epithelial cells of other species. The lack of a significant difference between the basally fed hamster tracheal epithelial cell cultures in Whitcutt chambers and the immersion-fed cultures in tissue culture dishes could have been because the hamster cells in culture are highly differentiated (11,13). Therefore, this study was extended to tracheal epithelial cells from other animals (rat, rabbit, guinea pig, and monkey) that exhibit different degrees of differentiation in culture, and to epithelial cells from human nasal and bronchial tissues. Again, in all these other species, except for the guinea pig, the method of nutrient provision did not affect the degree of differentiation. For instance, 20% of the high molecular weight secreted products from continuously immersed human bronchial epithelial cell cultures were hyaluronidase-resistant and mucinlike; changing the feeding method did not alter the level of differentiation. In contrast to these results, guinea pig tracheal epithelial cells were affected by the method of feeding. When fed basally, after being immersed 4 d in the culture medium, these cells differentiated to a mucociliary layer (Fig. 8 *a*) that was almost identical to mucociliary layers observed in vivo. Goblet cells appeared, replete with mucous granules of varying electron density (Fig. 8 *b*). This contrasted with the squamous and stratified appearance of the cultures that were constantly immersed in the culture medium (Fig. 8

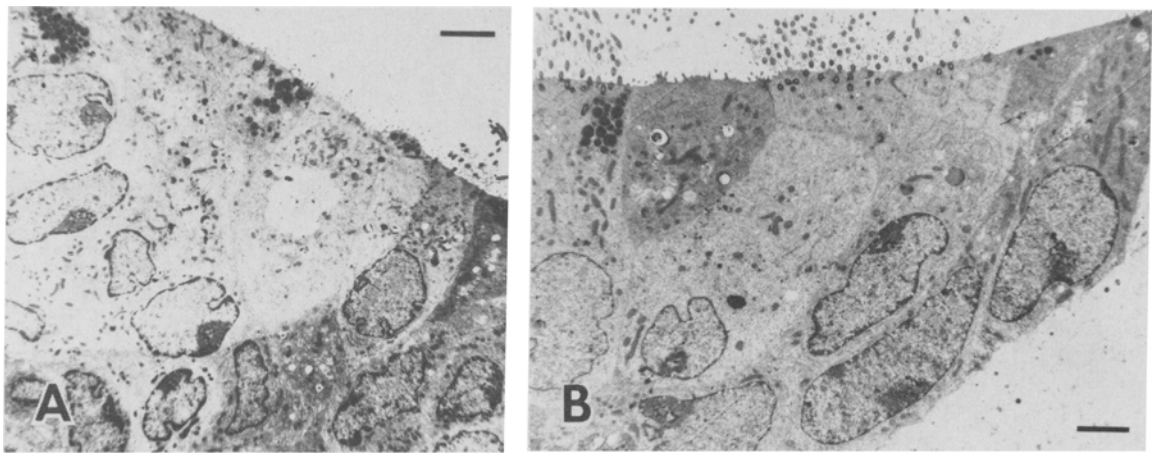
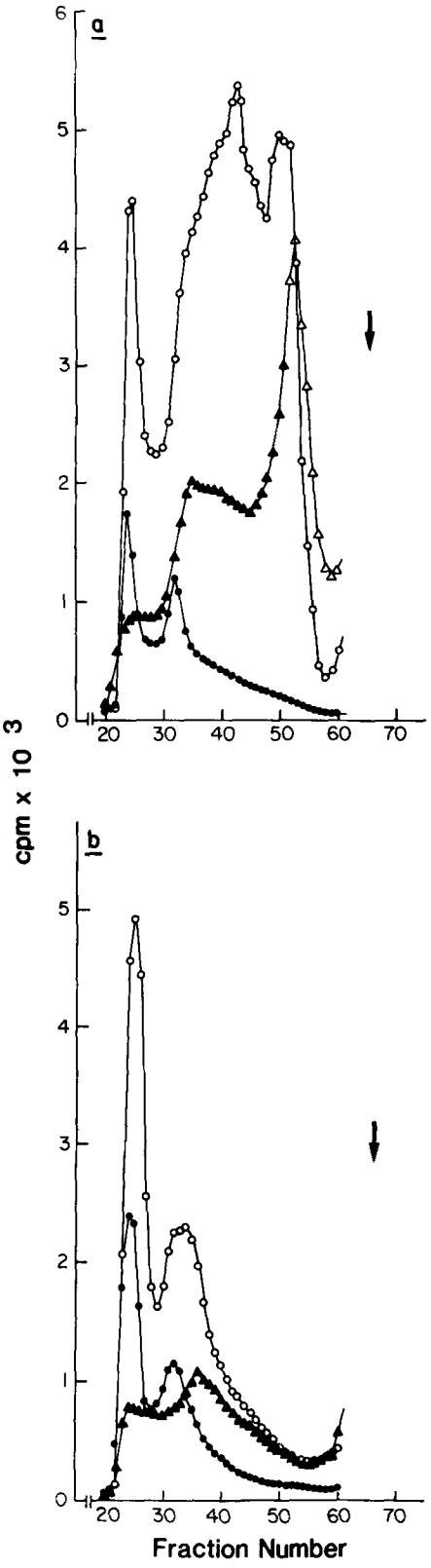


FIG. 6. Transmission electron micrographs of HTE cells cultured in the Whitcutt chamber. Cells were plated in the chamber, as described, in an immersed condition. Four days later, the feeding was changed and the cells were fed only from below. At Day 14, the cultures were fixed and examined by TEM. Note the columnar appearance (*B*) and differentiated features of the apical cells, such as cilia and mucous granules (*A*). *Bars* = 10 μm.

c). Thus, the method of feeding seems to play a vital role in the induction of differentiation of guniea pig airway epithelial cells.



Discussion

In this paper we have described the growth and differentiation of tracheal epithelial cells from various species, cultured under two different conditions: one is the traditional condition in which the cells are fed by the immersion method; the other is the new biphasic condition in which cells are maintained between air and liquid phases and fed from below (basal feeding). To achieve the latter conditions, a specialized culture system, the Whitcutt chamber, was developed. The chamber contains a movable, transparent, permeable gelatin membrane that can be used to support cell culture for basal feeding and biphasic cultivation. The membrane consists of a gelatin-coated nitrocellulose or polycarbonate filter which is mechanically supported by sandwiching the filter between a pair of nylon mesh spacers. The transparency of the filter is achieved by washing off the uncomplexed components of the filter with various solvents. The transparency of the filter in the Whitcutt chamber facilitates examination of living cells in culture. This is a clear advantage over some commercially developed chambers (e.g., Millicell from Millipore Corp., Bedford, MA). The diameter of the chamber, 25 mm in this study, can be expanded or reduced to any size. This flexibility will allow various biochemical, immunologic, and cytochemical studies on cells cultured in the Whitcutt chamber.

We have observed that the polarity of differentiation is better maintained in hamster tracheal epithelial cultures when these cultures are grown in the Whitcutt chamber and maintained in a biphasic condition. The quality of in vitro differentiation is similar to that of columnar tracheal epithelial in vivo, especially in areas of monolayer culture. Furthermore, differentiated features, e.g., mucus-secreting granules and cilia, are at the apical region. However, the expression of mucous-differentiated functions is not quantitatively affected by the use of this biphasic culture system.

This result may be due to the phenomenon that hamster tracheal epithelial cells can fully express differentiated functions under the traditional culture system (7,13). The appearance of columnar epithelia in some areas of culture under the biphasic condition suggests the importance of maintaining cultured airway epithelial cells in a condition similiar to that in vivo. In contrast, both the polarity and the mode of differentiation of guinea pig tracheal epithelial cells are affected by the choice of culture condition; only the biphasic culture method allows these epithelial cells to differentiate as in vivo. Although it is difficult to explain the

FIG. 7. Differential secretions of [^{3}H]glucosamine (*a*)- and [^{35}S]sulfate (*b*)-labeled molecules in HTE cells cultured under basal feeding conditions. Three different anatomic regions of the culture system were extracted and analyzed in a Sepharose CL-6B column. Lumen-associated (●), the rinse solution from the cell surface layer; cell-associated (○), extract from the cell layer after rinsing; medium-associated (▲), culture medium which was separated from the cells by a permeable membrane described in the text. *Arrows* indicate the peak fraction from which free radioactive precursors ([^{3}H]glucosamine, [^{35}S]sulfate) were eluted.

TABLE 1

EFFECT OF THE WHITCUTT CHAMBER CULTURE SYSTEM ON HAMSTER TRACHEAL MUCOUS CELL DIFFERENTIATION

Experiments	Time[b] in Culture, d	Percent PAS-positive population[a]	
		Whitcutt Chamber, basal feeding	Culture Dish, immersion feeding
I	2	0	1
	7	15	12
	10	25	31
	14	21	28
	21	16	20
II	2	3	2
	7	22	26
	11	39	41
	14	31	38
	21	25	35

[a]More than 200 cells from each dish or chamber were examined by the PAS method as described previously (13); the data presented represent the average of duplicate cultures.

[b]Change from immersion feeding to basal feeding in Whitcutt chamber cultures was performed on Day 4.

differences between species, these results suggest that, at least in the case of guinea pig, maintenance of respiratory tract epithelial cells under conditions similar to those in vivo, i.e. with the apical side exposed to air and with nutrients only available from the basal side, can improve their capacity for cell differentiation in vitro.

A second significant aspect of this study is the demonstration that all of the differentiated features, cilia and mucus-secreting granules, occur in the apical region of the epithelial layer when the cultures are fed basally. Results of biochemical analyses, which are consistent with the morphologic observations, indicate that mucin-like molecules are located in the apical region. The absence of these mucinlike molecules from the culture medium below is apparently not due to restrictions imposed by the membrane used in the Whitcutt chamber. The membrane is porous (Fig. 4) and allows the diffusion of macromolecules, such as peptide hormones, needed for cell growth. The absence of mucinlike molecules in the medium-associated compartment probably indicates that the phenomenon of differential secretion is an integral part of the cellular physiology of airway epithelial cells. Whether these differential secretions play important roles in determining cell differentiation will be the topic for further studies.

It is difficult to assess which one of the changes, the exposure to air, basal feeding, or others of still unspecified nature in the biphasic culture system, is involved in the elevation of differentiation of guinea pig tracheal cells. It is unlikely that the air contact plays any major role in the induction of cell differentiation because mature differentiation occurs in breathless fetal airway epithelial (17). However, basal feeding cannot totally account for the stimulation of cell differentiation in the biphasic culture system because it is equally difficult to rule out the possibility that basal feeding may occur in the traditional culture system, especially when the same kind of hydrated collagen gel substratum is used. At present, the nature of the improvement of cell differentiation in the Whitcutt chamber remains unresolved.

A floating collagen membrane culture system has been used previously to enhance mammary and thyroid epithelial cell differentiation (1,3,4). The study presented is this paper supports the notion that cell differentiation is enhanced when cells are cultivated on a floating system. However, despite the similarities between the two systems, the floating collagen gel system described for mammary and thyroid epithelial cultures does not seem to be suitable for studies of tracheal epithelial cells. Two problems are associated with the use of floating collagen gel substrate for these cells: a) some tracheal epithelial cells secrete a collagenaselike activity, which would remove all of the floating membrane; and b) some tracheal epithelial cells can phagocytose collagen gel. In the Whitcutt chamber, these problems are greatly reduced. Presumably, the mechanical support provided by the membrane in Whitcutt chambers and the basal feeding technique compensate for the potential instability of cultures resulting from collagenase degradation or phagocytosis.

In conclusion, the Whitcutt chamber described in this paper provides an in vitro system in which respiratory tract epithelial cells can be cultured between air and

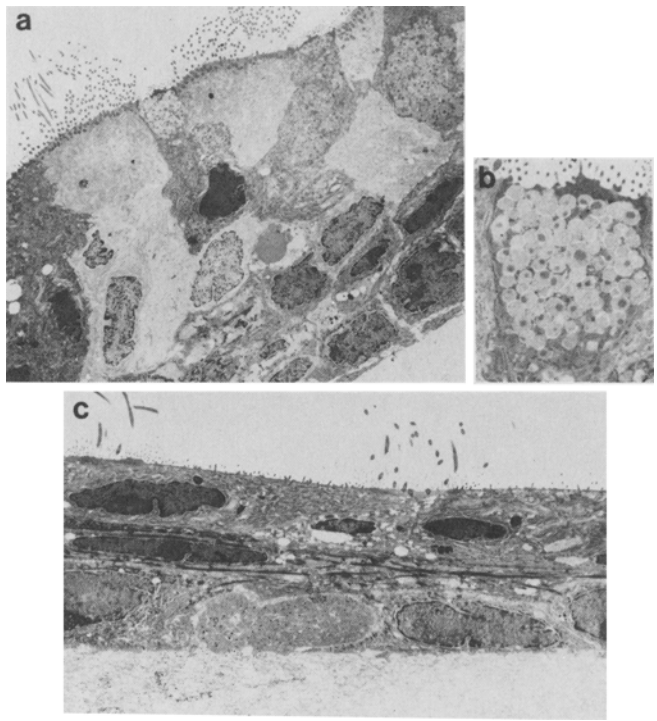


FIG. 8. Transmission electron micrographs of guinea pig tracheal epithelial cells cultured in Whitcutt chambers. *a*, cultures of basal feeding; *b*, enlarged picture of mucous granules in *a*; *c*, cultures of immersed feeding. As described in the text, basal feeding favored differentiation into a pseudostratified columnar epithelium virtually identical to that observed in vivo, whereas immersion feeding result in a stratified squamous configuration. *a*, ×2970; *b*, ×10 898; *c*, ×3883.

liquid phases. This system should be useful for studying normal and abnormal respiratory tract epithelial physiology. It should also find application in studies with other cells, e.g., skin, that exist in a similar three-dimensional organization in vivo.

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