THE PRIMARY CULTURE OF EPITHELIA FROM THE SECRETORY COIL AND COLLECTING DUCT OF NORMAL HUMAN AND CYSTIC FIBROTIC ECCRINE SWEAT GLANDS

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SUMMARY

Isolated human eccrine sweat glands have been microdissected into their secretory and reabsorptive components. Complete separation of these epithelia was confirmed by differential uptake of Neutral Red stain by an intermediate section of gland containing the junction between the secretory coil and the collecting duct. Primary cultures were obtained from explants of both tissues in medium RPMI-1640 or Williams E supplemented with foetal calf serum, insulin, transferrin, epidermal growth factor and hydrocortisone. The cells in the initial coil cultures had an elongated morphology while those of ductal origin were polyhedral. After 10 days both cultures were composed of polyhedral cells of varying diameter. All these morphological types were of epithelial lineage, as demonstrated by the binding of a monoclonal antibody to cytokeratin, the intermediate filament specific for epithelial cells.

Outgrowth from both secretory and reabsorptive epithelia were multilayered, with plentiful desmosomal connections and an underlying basal lamina. Ultrastructural features typical of the epithelial cell types present in intact eccrine sweat glands were absent in a high proportion of the proliferating cells but domes, indicative of transepithelial active ion transport, were present in dense cultures from the reabsorptive duct.

Outgrowth was also obtained from the secretory and reabsorptive epithelia of sweat glands from two cystic fibrotic patients. Since the most characteristic malfunction of cystic fibrosis is the impaired ion transport in the eccrine sweat gland, the availability of cultured epithelia should provide a useful model for study of the disease.

INTRODUCTION

The human eccrine sweat gland is an unbranched tubular structure composed of two functionally distinct epithelia: a proximal secretory coil, which secretes an isotonic fluid, and a distal collecting duct, which recovers NaCl from the sweat before it reaches the skin surface. Since the development of enzymic methods for the isolation of viable sweat glands in high numbers (Kealey, 1983; Okada et al. 1983), their relative simplicity of structure and function has made them attractive models for the study of secretion and reabsorption in exocrine glands.

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In addition, the malfunction of eccrine sweat glands is an important feature of the most common lethal inherited disease of Caucasian populations, cystic fibrosis. The disease has many potential manifestations, with abnormal exocrine gland secretions featuring prominently, but malabsorption and neonatal intestinal blockage caused by pancreatic insufficiency and the development of obstructive lung disease are the most severe clinical problems. In cystic fibrosis, the composition and volume of the sweat gland primary secretion is believed to be normal (Schulz, 1969), but impaired chloride reabsorption in the collecting duct, demonstrated in the microperfusion studies of Quinton (1983), is thought to cause a net reduction in both chloride and sodium transport out of the lumen.

The measurement of transport across tubular epithelia of narrow diameter presents many technical problems. However, the simpler techniques available for the measurement of transport across sheet epithelia, such as the frog skin, have been applied to epithelia of tubular origin after culture of the cells in flat sheets under conditions that permit the expression of their transport function. These include continuous lines such as MDCK and LL-PK1, of canine and porcine kidney tubule origin (Misfeldt et al. 1976; Misfeldt & Sanders, 1981), and primary cell lines such as mouse mammary and rat pulmonary alveolar cells (Bisbee et al. 1979; Mason et al. 1982).

The primary culture of cells from the secretory coil and collecting duct of the human sweat gland was undertaken: (1) in order to make available flat sheets of these epithelia to enable ion transport measurements to be made in Ussing chambers; (2) to overcome the restrictions of limited access to the lumen so that the activity of single channels can be measured by patch-clamping the apical as well as the basolateral membrane; (3) to increase the quantity of material available for biochemical analysis of normal and cystic fibrotic cells.

We report the separation of the secretory and reabsorptive portions of the sweat gland by microdissection, the primary culture of cells from explants of both tissues and the characterization of these cultures as epithelial by the binding of monoclonal antibodies to cytokeratin.

**MATERIALS AND METHODS**

**Materials**

The tissue culture media, foetal calf serum, tissue culture grade water and Hanks' balanced salt solution (HBSS) were purchased from Gibco (Europe) Ltd, Trident House, PO Box 35, Renfrew Rd, Paisley PA3 4EF, UK. Sodium benzylpenicillin and streptomycin sulphate were obtained from Glaxo Laboratories, Greenford Rd, Greenford, Middlesex UB6 0HE, UK. Amphotericin B and trypsin were obtained from Flow Laboratories, Woodcock Hill, Harefield Rd, Rickmansworth, Herts WD3 1PQ, UK. Insulin, transferrin, epidermal growth factor, hydrocortisone, collagenase type II and dianimobenzidine (DAB) were from the Sigma Chemical Co. Ltd, Fancy Rd, Poole, Dorset, UK. Anti-cytokeratin (PKK1, specific for cytokeratin filaments 8, 18 and 19, as defined by Moll et al. 1982) and anti-vimentin were obtained from Labsystems Ltd, 12 Redford Way, Uxbridge, Middlesex UB8 1SZ, UK; anti-desmin was obtained from Bio-Nuclear Services Ltd, 24 Westleigh Dr., Sonning Common, Reading RG4 9LB, UK, and peroxidase incubated rabbit anti-mouse immunoglobulin G (IgG) was from Dako Ltd, 22 The Arcade, The Octagon, High Wycombe, Bucks HP11 2HT, UK.
Isolation and microdissection of human eccrine sweat glands

Sweat glands were isolated from normal human skin as described by Lee et al. (1984), an adaptation of the method of Kealey (1983). Isolated glands were used either immediately after isolation or after overnight storage at 37°C in tissue culture medium containing 1% foetal calf serum, penicillin (100 units ml\(^{-1}\)), streptomycin (100 \(\mu\)g ml\(^{-1}\)) and amphotericin B (2.5 \(\mu\)g ml\(^{-1}\)) and buffered with 5% CO\(_2\):95% air. The glands were exposed to collagenase at 2 mg ml\(^{-1}\) in culture medium containing 5% foetal calf serum and antibiotics for varying amounts of time at 37°C. Following enzymic treatment, glands were incubated at 37°C in medium with 5% foetal calf serum for at least 30 min and transferred to phosphate-buffered saline (PBS), pH 7.3 (Dulbecco & Vogt, 1954), at room temperature immediately before microdissection.

The glands were illuminated with transmitted light to maximize the difference in appearance of the secretory and reabsorptive portions. Collagen fibres, which were occasionally wound loosely around the isolated glands, and adipocytes (when present) were removed using sterile micropins (A1, Watkins and Doncaster) attached to Pasteur pipettes with glass–metal glue. The collagen fibres binding apposing lengths of tubule were then gently removed to permit uncoiling of the gland and subsequent identification of the junction between the secretory coil and the collecting duct. The coil and duct were cut a short distance from this point, to ensure the complete separation of the two epithelial types, and removed. Neutral Red, which stains coil and duct differentially (Quinton, 1981), was then added to the junction to confirm that a length of each remained.

Tissue culture

The tissue culture medium was RPMI-1640 or Williams E, supplemented with penicillin (100 units ml\(^{-1}\)), streptomycin (100 \(\mu\)g ml\(^{-1}\)), amphotericin B (2.5 \(\mu\)g ml\(^{-1}\)), foetal calf serum (1%), insulin (10 \(\mu\)g ml\(^{-1}\)), transferrin (10 \(\mu\)g ml\(^{-1}\)), epidermal growth factor (10 ng ml\(^{-1}\)) and hydrocortisone (10 ng ml\(^{-1}\)), buffered with 5% CO\(_2\):95% air and used at 37°C. Medium containing supplements was not stored.

Two or three explants, either whole or halved sections of duct or coil, were placed on the bottom of a tissue culture flask (Primaria, Falcon) previously covered with 1 ml of tissue culture fluid at 37°C and gassed with a mixture of 5% CO\(_2\):95% air. After explantation, the flasks were left undisturbed at 37°C for at least 24 h and then gently tipped, to produce a flow of medium across the explants. After a further 24 h, 3 ml of medium at 37°C was added to the flask. Thereafter, the medium was changed every 3 or 4 days.

Removal of fibroblastic cell growth

In flasks where fibroblastic cell growth was apparent, cultures were washed in PBS and incubated with 5 ml of 0.25% trypsin in PBS for 5 to 10 min at 37°C. After the addition of 5 ml tissue culture medium containing 5% foetal calf serum, the rounded fibroblastic cells were removed by pipetting. The epithelial cells were washed twice in tissue culture medium and reincubated.

Light and electron microscopy

Cell outgrowth was routinely examined by phase-contrast using an inverted microscope (Diaphot, Nikon), with a 35 mm camera attachment. For electron microscopy, cells were fixed in phosphate-buffered glutaraldehyde (3%), pH 7.3, post-fixed in isotonic osmium tetroxide (1%) and embedded in Araldite. Ultrasections (prepared on an LKB Ultratome 111) were double stained with uranyl acetate and lead citrate, and examined in an AE1 Corinth 275 electron microscope.

Immunocytochemistry

Frozen sections of whole skin (8–10 \(\mu\)m thick) were air-dried on microscope slides for 30 min at room temperature before fixation. Unfixed frozen sections of appropriate tissues were used as controls for cytokeratin, desmin and vimentin labeling. Cultured epithelia were washed in Ca\(^{2+}\)/Mg\(^{2+}\)-free PBS (PBSA), incubated with 0.02% EDTA in PBSA for 5 min at 37°C and
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0.02% EDTA/0.25% trypsin in PBSA for 15 min at 37°C, pelleted and resuspended in HBSS. The cells were cytospun onto slides and air-dried before fixation.

Samples tested using anti-cytokeratin and anti-desmin were fixed in acetone at room temperature for 15 min; samples tested with anti-vimentin were fixed in methanol/chloroform/acetic acid (6:3:1, by vol.) at —20°C for 5 min. An indirect immunoperoxidase technique was applied using DAB to develop peroxidase. Sections were counterstained in Mayer's haemalum, dehydrated in ethanol, cleared in xylol and mounted in resin.

RESULTS

The identification of secretory coil and collecting duct

Fig. 1A shows an uncoiled sweat gland. Electron microscopy of the larger diameter segment (Fig. 1B) showed the characteristic light and dark cells of the secretory coil. The more sharply defined tube of narrower diameter (Fig. 1C) contained the luminal and serosal cells of the duct (see Munger, 1961, for a review of cell types). The difference in the light microscopic appearance of the two epithelial types was enhanced by a low level of transilluminating light directed towards the specimen at an oblique angle.

Sweat glands from the same individual were relatively uniform in appearance but varied between subjects in: (1) the total length of the unwound tubule (approx. 1–9 mm); (2) the relative proportion of coil to duct (approx. 25–75%, by length); and (3) the diameter of the coil relative to that of the duct (approx. ×1 to ×3). Infrequently, glands were isolated in which coil and duct did not differ sufficiently in texture or diameter to be distinguished without staining. These were not used for tissue culture experiments.

Separation of secretory coil from collecting duct by pulling resulted in a short segment of secretory epithelium remaining attached to the duct. For this reason, each gland was microdissected into three sections: secretory coil, reabsorptive duct and a short section of tubule containing the junction between the two. After removal of the coil and duct sections, Neutral Red added to the junctional segment was uniformly taken up by the cells of the secretory epithelium but was concentrated in the duct lumen, as described by Quinton (1981).

The effect of collagenase treatment

Coils and ducts were successfully explanted without exposure to collagenase. The proportion of non-enzymically treated explants that resulted in outgrowth varied from patient to patient, but was never 100%. Limited treatment with collagenase made the teasing apart of glands much easier and increased the incidence of outgrowth of both coil and duct explants to 100%. However, prolonged digestion caused partial dissociation of the secretory coil. Although the intact and more elastic duct was therefore easily removed from the coil, digestion was not allowed to proceed to this stage because of the possibility that dispersed secretory coil cells would adhere to the duct, resulting in an explant of mixed cell types. The optimum time for collagenase treatment was usually around 30 min.
Fig. 1. A. An eccrine sweat gland isolated from a 43-year-old male, unwound before microdissection. The slightly granular appearance of the secretory coil (sc) contrasts with the more sharply defined collecting duct (cd). The junction (j) between the two epithelia may also be marked by an abrupt narrowing, since the secretory coil diameter may be as much as threefold greater than that of the collecting duct. B. A transverse section of the wider diameter tubule showing the dark and light secretory cells of the secretory coil. The dark cells (d) bordering the lumen contain large numbers of membrane-bound secretory granules and are typical of mucigenous cells. The light cells (l) that occasionally border the lumen are thought to be responsible for serous secretion. Myoepithelial cells (not shown) are present in the basal region. C. A transverse section through the wall of the smaller diameter tubule showing the characteristic concentric arrangement of collecting duct cells. The cytoplasm of the inner cells contain large numbers of tonofilaments, particularly around the lumen (lu). These keratin filaments are a feature of supportive epithelia and are believed to act by distributing mechanical forces evenly throughout the cell. The borders of adjoining serosal cells are intermeshed with the long villous processes common in fluid transporting epithelia. A, ×44; B,C, ×6000.
Fig. 2. Outgrowth from sweat gland secretory coil: A, 24 h after explantation; B, 3 days after explantation, showing cells of polygonal morphology with long cytoplasmic processes and slightly larger cells without these extensions; and C, 26 days after explantation, showing a compact cobblestone appearance. Cells cultured for this time show accumulations of phase-bright cytoplasmic granules. A, ×50; B, ×175; C, ×44.
Storage of sweat glands

Glands with a minimal amount of contaminating collagen could be stored for up to 3 days in a medium containing 1% foetal calf serum. After this time the increased exposure to collagenase required to facilitate unwinding caused breakdown of the secretory coil. The incidence of successful outgrowth from explants of glands was unchanged after storage in Williams E but was reduced after storage in RPMI-1640.

Morphology of secretory coil cultures

Initial culture experiments were performed in RPMI-1640. However, when it was found that proliferative capacity was retained more effectively after storage in Williams E, the effects of similarly supplemented RPMI-1640 and Williams E were compared. There was no observed difference between media with respect to the time at which migrating cells first became apparent or in the subsequent pattern of epithelial outgrowth.

Fig. 2A shows the outgrowth obtained from a secretory coil fragment 24 h after explantation. The cells originate from the entire length rather than the cut ends of the epithelium and are aligned perpendicularly to the longitudinal axis. They migrate a short distance from the explant before dividing, giving rise to a culture that is initially loosely packed. The majority of cells in the early phase of secretory coil outgrowth are triangular, with apices extended in relatively long cytoplasmic processes, which give them an elongated appearance (Fig. 2B). After 7 days in culture, the outgrowth consisted predominantly of compact polygonal cells of varying diameter, with larger cells apparently underlying this population. Phase-bright cytoplasmic granules were apparent after about 2 weeks of culture and multinucleate cells were observed after approximately four weeks. Fig. 2C shows part of a 26-day outgrowth with the ‘cobblestone’ appearance characteristic of epithelial cells in culture. Outgrowths of approximately 3 cm diameter, obtained from single coil explants, contained 7.7×10⁵ cells (±1.41, n = 2). A count of 5.6×10⁵ (±0.88) cells per gland was obtained after dissociation of whole glands in batches of 10 (n = 2), confirming that proliferation had occurred in vitro.

Electron micrographs of proliferating secretory coil cultures showed them to be multilayered. The cells were flattened when grown on plastic and tight junction formation was not apparent; however, they demonstrated a polarized deposition of basal lamina and desmosomal connections. Although cells containing dark granules or concentrations of filaments were occasionally seen, most cells were not readily identifiable as light, dark or myoepithelial. Fig. 3 is a typical cross-section, showing flattened cell layers with desmosomal connections. The basal cell has dilated endoplasmic reticulum and sits on a basal lamina. Dark granules are seen in a cell nearer the apical surface.

Morphology of collecting duct cultures

The outgrowth obtained from a duct fragment incubated overnight is shown in Fig. 4A. As with the coil cultures, there was no apparent difference between those
Fig. 3. An electron micrograph of a cross-section of sub-confluent secretory coil culture 18 days after explantation, showing a multilayer of flattened cells linked by desmosomes. The cell in contact with the culture dish contains dilated rough endoplasmic reticulum and has an adherent basal lamina. Occasional dark granules are seen in apical cells. ×6000.

grown in RPMI-1640 or Williams E. Duct cells also grew out from the length of the explant rather than exclusively from the cut ends. However, migration was not as extensive as seen from the coil; the earliest cells to move outwards from the duct explant retained contact with the tissue and grew closely apposed to each other. The cells comprising the early culture were small, with characteristically epithelial morphology and clearly defined phase-bright boundaries (Fig. 4B). However, after several weeks, cultures originating from the reabsorptive duct (Fig. 4C) were morphologically indistinguishable from those of secretory coil origin (Fig. 2C). The final diameter of outgrowths from duct explants varied between batches of glands but were usually less than the equivalent coil explants.

The micrographical appearance of proliferating duct cultures was very similar to that of coil cultures, with flattened cells growing in a multilayer, basal lamina formation and desmosomal connections (Fig. 5A). However, differentiated features were more readily identifiable in the duct cells, with concentrations of tonofilaments in the cytoplasm and extensive microvilli on the apical surface (Fig. 5B). Although tight junctions were not seen by electron microscopy of reabsorptive duct outgrowths, domelike structures were apparent in outgrowths of greater than 2 cm diameter (Fig. 5C).

Removal of fibroblastic cell growth

Fibroblastic cell growth was observed at the edges of the epithelial outgrowth in a proportion of cultures, which was usually less than 50%, but varied from batch to batch. The fibroblastic cells became apparent at varying times after day 10 and were more often seen in coil explant cultures than in duct. Elimination of fibroblastic growth was achieved by repeated trypsinization at 3-day intervals.
Fig. 4. Outgrowth from sweat gland reabsorptive duct: A, 24 h after explantation, showing a tightly packed outgrowth of polygonal cells contrasting with that of secretory coil; B, 3 days after explantation; and C, 25 days after explantation, showing polygonal cells of varying size. Phase-bright cytoplasmic inclusions indicative of cell senescence are apparent in many cells. A, ×60; B, ×175; C, ×44.
Immunocytochemical characterization

Sweat gland epithelia in situ were positively labelled with anti-cytokeratin (Fig. 6A) and negative when tested with desmin and vimentin.

Cells from fibroblast-free coil and duct cultures obtained from the sweat glands of three patients were tested after 10 days outgrowth. All cells were strongly positive for cytokeratin (Fig. 6B, C) and negative for desmin. Approximately 5% of the coil cells were also positive for vimentin. On one occasion, coil outgrowths from the same batch of glands were tested on day 7 and day 13. The proportion of cells expressing vimentin did not appear to have altered during this time. Duct cell cultures were not tested for vimentin. The lineage of the cells with extended processes seen in early coil

Fig. 5. Sub-confluent duct cell cultures are shown to be multilayered with desmosomal connections and a basal lamina (shown here detached) laid down on the culture support (A). Occasionally cells show differentiated features such as tonofilaments in the cytoplasm and apical microvilli (B). In densely packed duct cultures (C) dome formation, indicating the active transport of fluid in an apical to basolateral direction, is evident. A, x10000; B, x6000; C, x30.
Outgrowth from glands of cystic fibrotic origin

Cultures were initiated from the secretory and reabsorptive epithelia of sweat glands isolated from two cystic fibrotic patients. The phase-contrast appearance of
the outgrowth from the coil explants was similar to that of normal tissue. However, holes in the cultures (Fig. 7A) and phase-bright intracellular inclusions (Fig. 7B), indicative of ageing cultures, were apparent slightly earlier than normal. The initial outgrowth of cystic fibrotic ductal cells (Fig. 8A) was apparently the same as that from ducts of normal origin (Fig. 4A,B) and there was no evidence of earlier ageing in cystic fibrotic duct cultures (Fig. 8B) compared to normal (Fig. 4C).

Fig. 7. Secretory coil cultures from sweat glands of cystic fibrotic origin, 15 days after explantation. The morphological appearance of the cells was similar to that of cultures from normal controls except that holes appeared in the cell sheet after approximately 10 days (A), and phase-bright granules (B), began to accumulate approximately 5 days earlier than normal. A, ×30; B, ×150.
Fig. 8. Reabsorptive duct cultures from sweat glands of cystic fibrotic origin on: A, day 3; and B, day 20. The morphology of the cells and the rate and extent of outgrowth is similar to that of cultures from normal reabsorptive duct. A, ×100; B, ×110.

DISCUSSION

Medium RPMI-1640 was initially chosen as a basal medium since it was known to support all of the sweat gland cell types in a healthy state during organ maintenance
CM. Lee, F. Carpenter, T. Coaker and T. Kealey (Lee et al. 1984). The reason why the proliferative capacity of both coil and duct is maintained more effectively on prolonged storage in Williams E than in RPMI-1640 is unknown, and was not investigated.

Immunological labelling of the intermediate filaments of cultured coil and duct cells with antibodies to cytokeratin confirmed their lineage as epithelial. Intermediate (10 nm) filaments are present in almost all vertebrate cells and form, together with actin-containing microfilaments (6 nm) and tubulin-containing microtubules (23 nm), the basis of the cytoskeletal framework. They are composed of five major classes of protein (cytokeratin, neurofilaments, glial fibrillary acidic protein, desmin and vimentin), which are specific for epithelial, neuronal, glial, muscle and mesenchymal cells (such as fibroblasts, macrophages and endothelia), respectively (Moll et al. 1982). The co-expression of cytokeratin and vimentin has been previously reported in epithelial cell cultures (Franke et al. 1979; Osborne & Weber, 1983). The induction of vimentin expression in vitro has not yet been satisfactorily explained.

Phase-contrast examination of the primary cultures showed that the epithelial cells were of mixed morphological types. The morphologically distinct populations could not be correlated with the epithelial types present in the original tissue, since examination by electron microscopy showed that a high proportion of the cultured cells did not evidence the markers that typify the sweat gland cells in situ, e.g. the secretory granules of dark cells in the coil and the concentration of tonofilaments in duct luminal cells. Although several specialized features of epithelia, such as desmosomal connections and polarity of basal lamina deposition and microvillar formation, had been retained in the outgrowing cultures, it is probable that many differentiated characteristics are not expressed by cells in the proliferative phase. Cells that did show specialized ultrastructural features may represent a smaller population of differentiated, non-proliferating cells.

Tight junctions were not identified in cultures of either secretory or reabsorptive epithelia, but dome formation was observed in densely growing cultures from collecting duct. Dome formation is due to the detachment of cells from an underlying support by the accumulation of fluid transported through the cell sheet and is dependant on the maintenance of plasma membrane polarization, the integrity of occluding junctions and the expression of transepithelial active ion transport (reviewed by Lever, 1984). Although basolateral to apical ion transport, the function of the secretory coil in vivo, is not demonstrable by such indirect means, primary cultures generally do retain the capacity to express differentiated functions when the appropriate environmental conditions are provided (for a brief review, see Freshney, 1983).

Using eccrine sweat glands isolated by collagenase digestion of human skin, Pedersen (1984) has recently obtained outgrowth of cells from explanted collecting duct in Ham's F12 medium. No confirmation of the separation of duct from coil before explantation was reported and no attempt was made to detect non-epithelial cell types in the culture. However, the formation of domes was observed in the cell sheet, indicating that functional reabsorptive cells were present.
Culture of epithelia from eccrine sweat glands

The successful propagation of the secretory and reabsorptive epithelia of the normal sweat gland described here provides a model for the study of transepithelial transport, which should usefully complement studies performed on the intact tissue. In addition to providing a means of examining the nature and control of vectorial transport under the readily manipulated environment of an Ussing chamber, permitting access to the luminal surface of the cells and obtaining increased quantities of material, tissue culture also provides an opportunity to observe the cells in an actively proliferating (sub-confluent) phase as well as a non-proliferating, functionally differentiated state (confluence), which most closely resembles that of the intact gland.

The confirmed separation of coil and duct epithelia, the characterization of the population as entirely epithelial in origin and the limited number of cell types present in the initial tissues (three in the secretory coil and two in the reabsorptive duct) mean that the cultures are of narrowly defined origin. They may therefore provide a useful starting point from which to study the role of individual cell types in the respective transport processes.

We have demonstrated the fibroblast-free culture of epithelia from sweat glands of cystic fibrotic origin. On the basis of a limited study it is difficult to interpret the observation that the cystic fibrotic secretory coil cultures appeared to age more rapidly than normal. Although this may be attributable to an inherent defect in the cells, it is likely that some tissues from ill subjects may simply have a reduced proliferative capacity. There have been several reports of impaired ion transport across epithelia in cystic fibrosis: in the reabsorptive duct of the eccrine sweat gland (Quinton, 1983), respiratory epithelia (Knowles et al. 1981) and the placenta (Davis et al. 1985). However, the most characteristic abnormality is that of the sweat gland, which results in an increase in NaCl concentration in sweat that is diagnostic for the disease (Di Sant'Agnese et al. 1953). The availability of cultured epithelia from a tissue known to express the transport malfunction should provide a valuable tool for cystic fibrosis research.

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