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A new method for studying human polycystic kidney disease epithelia in culture

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A new method for studying human polycystic kidney disease epithelia in culture. Human polycystic kidney disease (PKD) epithelia were successfully grown in culture and expressed abnormal characteristics. Cysts lining epithelia of superficial and deep cysts were microdissected and compared to individual normal human proximal straight tubules (PST) and cortical collecting tubules (CCT) grown in defined media. PKD cyst epithelia differed from normal renal tubular epithelia in growth patterns and structural and functional properties. PKD epithelia grew more rapidly and showed cyst–like areas in otherwise confluent monolayers. Polygonal and elongate cells contained an epithelial–specific cytokeratin antigen and had polarized morphology. An extremely abnormal basement membrane morphology was seen and consisted of some banded collagen and numerous unique blebs or spheroids. These blebs were apparently extruded from intracellular vacuoles and stained with ruthenium red, suggesting a proteoglycan component. Cytochemistry of marker enzymes demonstrated the presence of NaK-ATPase and alkaline phosphatase, but a lack of γ-glutamyl transpeptidase. The response of adenylate cyclase activity to vasopressin, parathyroid hormone, and forskolin was significantly diminished in PKD cells as compared to PST and CCT. These studies suggest a defect in cell growth and basement membrane synthesis in human PKD. Cultured PKD epithelia provide a new tool for the study of the pathogenesis of this disease.

Autosomal dominant polycystic kidney disease (PKD) is a major cause of end–stage renal failure and is the most common heritable renal disease in the dialysis and renal transplant patient population. There are few data on the pathogenesis of PKD although several theories have been proposed. Current opinion favors the possibility of either the existence of an abnormal basement membrane [1] or abnormal tubular epithelial growth [2]. Recent awareness of the important role of the extracellular matrix in control of epithelial cell growth, migration, shape, and polarity may lead to the conclusion that these two theories are not mutually exclusive.

The suggestion of an abnormal basement membrane in human PKD has been provided by some electron microscopy studies [1–3]. The apparent systemic nature of the disease suggested by concurrent formation of cysts in other organs and cardiovascular abnormalities also suggest a disorder of the extracellular matrix [4, 5]. Structural and biochemical changes in the basement membrane have also been described in an animal model of drug–induced cystic disease [6, 7]. Since the basement membrane has been shown to be responsible for maintaining the distensibility of renal tubules [8], a change in the relative proportions of collagenous, non-collagenous glycoprotein, and proteoglycan components would account for the observed increased compliance of PKD epithelia, and thus would contribute to cyst formation.

To effectively determine the pathogenesis of human PKD, it would be highly advantageous to isolate and culture, in parallel, human cystic and normal renal epithelia to provide sufficient cells to analyze possible structural, functional, and biochemical defects in both the cells themselves and in extracellular matrix produced by those cultures. Since normal human renal epithelia of proximal straight tubule (PST), thick ascending limb of Henle's loop (TAL) and cortical collecting tubule (CCT) origin have been successfully grown in culture and shown to retain differentiated characteristics [9] similar techniques were applied to determine whether human PKD epithelia could be cultured with the view to study abnormalities in growth, morphology, cellular and basement membrane ultrastructure, enzyme activities, and hormone response.

Methods

Microdissection of normal and polycystic kidneys

Polycystic kidneys removed from eight PKD patients with end–stage renal failure and 14 normal human cadaver kidneys, the latter being judged unsuitable for transplantation, were microdissected. The kidneys were from individuals ranging in age from 13 to 38 years. The procedure for microdissection of individual segments of normal human kidney PST and CCT has been described in detail elsewhere [9]. Briefly, thin renal cortical slices were washed three times in dissection media consisting of RPMI 1640 [10] (Irvine Scientific, California, USA) containing high doses of the antibiotics penicillin (600 U/ml) and streptomycin (600 μg/ml), and then individual PST and CCT separated from medullary rays using finely sharpened watchmaker's forceps and a dissecting microscope (Bausch and Lomb). Approximately 10 individual PKD cysts of superficial (PKDs) and 10 of deep (PKDd) origin were individually dissected out from each polycystic kidney. These were stripped clean of fibrous tissue to leave the epithelial layer, cut open and washed extensively in RPMI media, pH 7.4, containing high doses of antibiotics as described above. Irrespective of original cyst size, lining epithelia were carefully chopped into approximately

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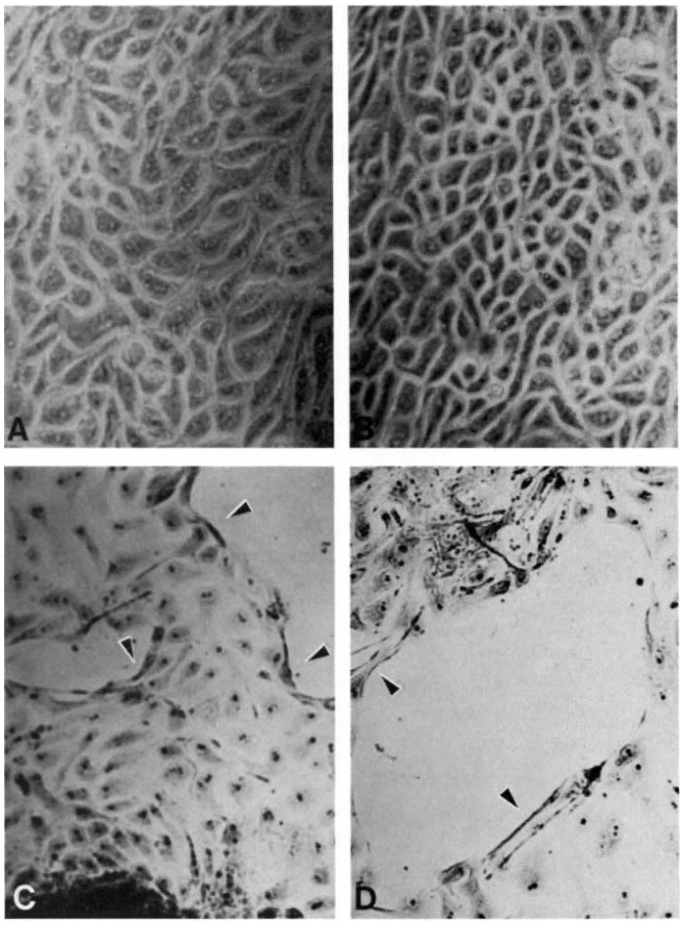


Fig. 1A. *Light micrograph of confluent PST monolayer derived from a normal human kidney.* Phase contrast optics. (×106) **B.** Light micrograph of confluent CCT monolayer derived from a normal human kidney. Phase contrast optics. (×106) **C.** Light micrograph of confluent PKDs monolayer showing polygonal and elongate cells (↑) lining "cyst-like" area. Formalin fixed and methylene blue stained. (×106) **D.** Light micrograph of confluent PKDd monolayer showing polygonal and elongate cells (↑) lining large "cyst". Formalin fixed and methylene blue stained. (×106)

1 mm^2 pieces using a scalpel. No collagenase was used in either microdissection.

Culture

Approximately 50 nephron segments (PST and CCT) from normal kidneys and 50 pieces from 10 cysts were explanted individually onto tissue culture plastic, 35 mm, (Falcon, California, USA) and gas-permeable Teflon membranes, 60 mm, (Heraeus) coated with a thin film of dried 20% collagen (Ethicon). Each piece of tissue was touched down in 100 μl of appropriately supplemented tissue culture medium, pH 7.4, 290 mOsm, and incubated for 1 hr at 37°C in humidified 5% CO_2 95% air before gently feeding with sufficient medium to cover the dish floor. Approximately 90% of tubules and cysts subsequently grew in culture. The media used for culture were RPMI 1640 containing sodium bicarbonate (24 mM), Hepes buffer (10 mM), penicillin (100 U/ml), streptomycin (100 μg/ml), human transferrin (5 μg/ml), and fetal calf serum (FCS, 3%, Hazleton, Pennsylvania, USA). Additional hormone supplements added were dexamethasone (10^{-8} M) for CCT and PKDd and dexamethasone (10^{-8} M) plus insulin (50 ng/ml) for PST and PKDs. After 15 to 25 days of culture, FCS could be removed from the media without damaging the integrity of the cellular monolayer.

Light microscopy

Cell monolayers were viewed under an inverted microscope (Nikon IM 35) either unstained using phase optics or after 5 min fixation in 10% formalin and 5 min staining with methylene blue.

Transmission electron microscopy

Discrete sections of polycystic kidney, whole cysts and confluent PST, CCT, PKDs, and PKDd monolayer cultures grown on Teflon membranes were fixed for 1 to 4 hr at 4°C in 2% glutaraldehyde in 100 mM sodium cacodylate buffer, pH 7.2, containing 5% sucrose, washed overnight in cacodylate buffer, postfixed for 1 hr in 1% osmium tetroxide, dehydrated in graded ethanols and embedded in Araldite resin. Intact pieces of polycystic kidney were also fixed in 3% glutaraldehyde for 4 hr at 4° and processed in an identical fashion for electron microscopy. Ultrathin sections were poststained with uranyl acetate and lead citrate and viewed under a Philips 300 electron microscope. Ruthenium red (2 mg/ml) was added to the fixative of some cells [11] to selectively stain extracellular matrix glycoproteins and proteoglycans.

Enzyme cytochemistry

Confluent, growth arrested monolayer cultures of 4 to 8 PST, CCT, PKDs, and PKDd washed twice for 3 min at 4°C in phosphate buffered saline (PBS), frozen rapidly in isopentane over dry ice, and frozen sections of freshly isolated PKDs and PKDd were incubated for 45 min at 25° or 37°C in one of the following media:

Alkaline phosphatase. 100 mM Tris-maleate buffer, pH 9.4, 1 mM beta-glycerophosphate (disodium salt, Sigma, St. Louis, Missouri, USA), 4 mM magnesium sulfate, and 3 mM alkaline lead citrate followed by treatment with 1% ammonium sulfide [12].

γ-Glutamyltranspeptidase. 50 mM sodium phosphate buffer, pH 7.4, 0.126 mg/ml γ-glutamyl-4-methoxy-beta-naphthylamide (Sigma), 0.5 mg/ml glycyl-glycine, and 0.5 mg/ml fast blue BBN followed by post-coupling with 100 mM copper sulfate [13].

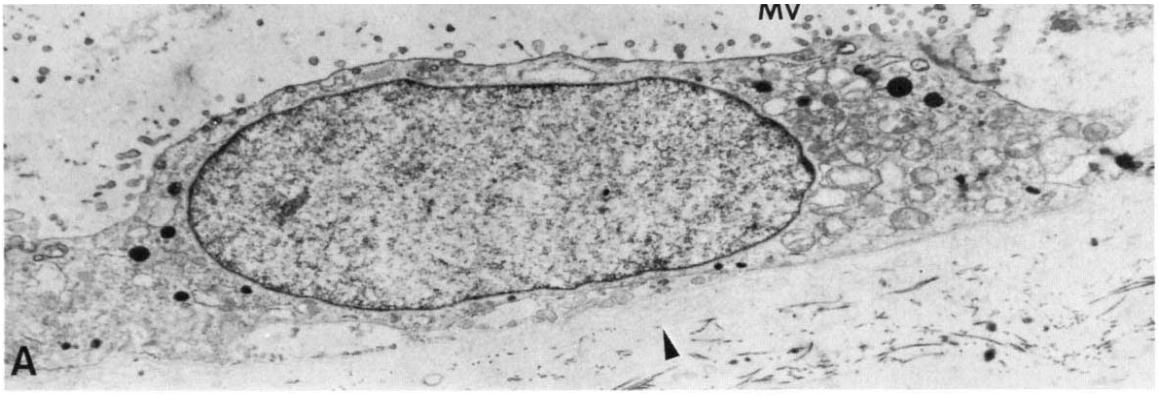
NaK-ATPase (K-dependent Phosphatase). 100 mM Tris-HCl buffer, pH 9.0, 20 mM sodium p-nitrophenyl phosphate (Sigma), 200 mM magnesium chloride, 10 mM potassium chloride, 25% dimethylsulfoxide, and 30 mM strontium chloride followed by post-coupling with 2% lead nitrate and visualization with 1% ammonium sulfide [14].

Cytochrome oxidase. 100 mM sodium cacodylate buffer, pH 7.4, 1 mg/ml cytochrome, 1 mg/ml 3,3'-diaminobenzidine-4 HCl, and 2 μg/ml catalase [15].

After incubation, cell cultures were washed in appropriate cytochemical buffer, fixed for 5 min in 2% formaldehyde, rinsed in 100 mM cacodylate buffer and air dried.

Immunocytochemistry of cytokeratin

A monoclonal antiserum (LE61) known to immunolocalize keratin intermediate filaments in all renal tubular cells was employed [16]. Confluent monolayers of PST, CCT, PKDs, and



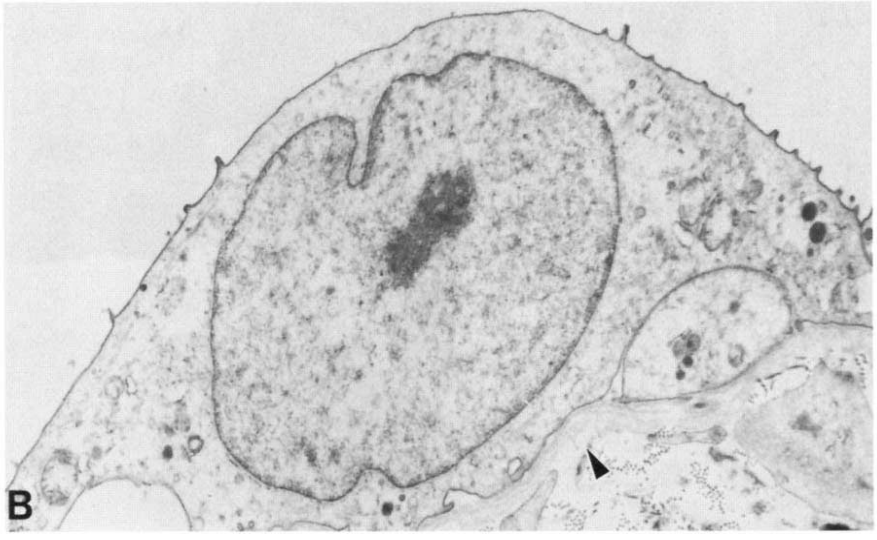


Fig. 2A. *Electron micrograph of PKD proximal cyst lining epithelial cell from intact cyst.* The flat cell lies on a thickened basement membrane (↑) containing some collagen fibrils. Apical microvilli (MV), abundant mitochondria, lysosomes and microbodies are seen. (×12,000) **B.** Electron micrograph of PKD distal cyst lining epithelial cell from intact cyst. The flat cell lies on a thick and laminated basement membrane (↑) and contains few mitochondria, lysosomes and apical microvilli. (×12,000)

PKDd and frozen sections of freshly isolated PKDs and PKDd were fixed for 5 min at −20°C in 20% methanol, washed with PBS, and incubated with pre-immune blocking serum prior to 45 min at 25°C incubation with LE61 antiserum. Cytochemical visualization of the peroxidase end–product was achieved using an avidin–biotin technique [17].

Adenylate cyclase biochemical assay

Cell monolayers and freshly isolated cysts were washed twice for 3 min at 4°C with PBS, protein content determined [18], and samples permeabilized by freezing and thawing in hypoosmotic solution (0.025 mM EDTA, 1 mM $MgCl_2$, 0.1% bovine serum albumin, 1 mM Tris-HCl, pH 7.4). Adenylate cyclase was measured using a modification of the method of Morel [19]. Cultured cells were incubated for 30 min at 37°C in 5 μl medium containing 3.8 mM $MgCl_2$, 1 mM cAMP, 0.25 mM EDTA, 20 mM creatine phosphate, 1 mg/ml creatine phosphokinase, 0.25 mM alpha-^{32}P-ATP (4×10^6 cpm) and 100 mM Tris-HCl, pH 7.4. Reaction was stopped with 150 μl ice–cold 50 mM Tris-HCl, pH 7.6, containing 3.3 mM ATP, 5 mM cAMP, and 10,000 cpm ^{3}H-cAMP for recovery determination. Produced ^{32}P-cAMP was separated by two–step elution over Dowex-50 and alumina columns [20]. Activity was measured in the presence and absence of arginine vasopressin (10^{-6} M), parathyroid hormone (10 U/ml), and forskolin (25 μM).

Results

Growth

PKD epithelia in culture started to proliferate after five to seven days and reached confluence more rapidly (20 to 24 days) than normal PST and CCT epithelia cultured under similar conditions (35 to 41 days). In contrast to normal PST and CCT which formed monolayers of tightly packed polygonal cells (Figs. 1A and 1B), PKDs and PKDd monolayers possessed an average of four (range 2 to 10) areas denuded of cells and lined by extremely elongate cells, although the majority of the culture consisted of polygonal cells (Figs. 1C and 1D).

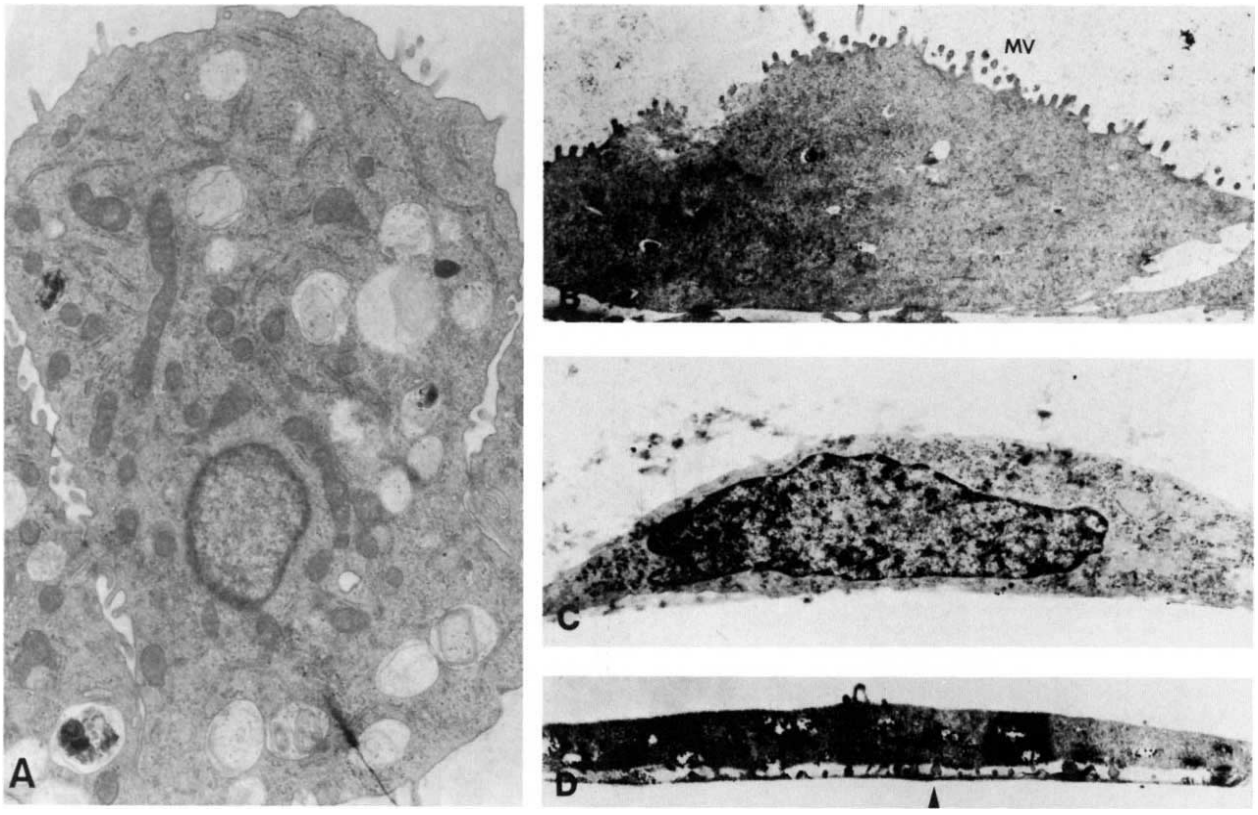


Fig. 3A. *Electron micrograph of PST cell cultured from a normal kidney showing abundant mitochondria and rough endoplasmic reticulum and some apical microvilli.* (×12,000) **B.** Electron micrograph of polygonal PKD cell showing a flattened nature, numerous, short apical microvilli (MV), rough endoplasmic reticulum, and free ribosomes. (×8,400) **C.** Electron micrograph of CCT cell cultured from a normal kidney showing abundant rough endoplasmic reticulum and free ribosomes. (×8,400) **D.** Electron micrograph of elongate PKD cell showing extremely flattened nature, abundant free ribosomes, intracellular vacuoles, and extracellular basal blebs (↑). (×8,400)

Ultrastructure

Sections of polycystic kidneys and cysts demonstrated lining epithelia which were characteristically flattened cells with short apical microvilli lying on an often thickened basement membrane (Fig. 2). Cells of presumed proximal tubule origin contained numerous mitochondria, lysosomes and microbodies (Fig. 2A). Other cysts were lined by cells that possessed few cellular organelles, few short apical microvilli, and had short intracellular occluding junctions suggesting a collecting tubule origin (Fig. 2B).

Cultured PKD cells, like cultured PST and CCT cells, showed a polarized ultrastructure with apical microvilli facing the medium and basal surfaces adjacent to the dish (Fig. 3). In each PKD culture, many cells were flattened by comparison to cultured normal PST and contained more numerous short apical microvilli, fewer mitochondria, and lysosomes, but equally abundant rough endoplasmic reticulum (Figs. 3A and 3B). Other PKD cells in culture were extremely flattened in comparison to cultured normal CCT, and contained few microvilli or organelles, but displayed many unique intracellular vacuoles not seen in normal cultured renal epithelia (Figs. 3C and 3D).

The most striking difference in ultrastructure between normal cultured epithelia and PKD epithelia was seen in the basement membrane morphology (Fig. 4). Normal PST and CCT had a thin layer of basement membrane material adjacent to their basal surfaces, composed of an electron lucent (lamina rara) component immediately adjacent to the cell and a thin electron dense (lamina densa) component (Figs. 4A and 4C). Cultured PKDs and PKDd cells had unique abnormal "blebs", or spherical aggregates of proteinacious material, and bundles of banded collagen in association with their basal surfaces (Figs. 4B and 4D). These features were never seen in the extracellular matrix of normal cultured renal epithelia. These blebs or spheroids were also present in vacuoles within a PKD cell (Fig. 5) and stained lightly with ruthenium red (Fig. 6), suggesting a proteoglycan composition [21].

Cytochemistry

The semiquantitative analysis of enzyme and keratin staining in cultured PKDs, PKDd, and normal PST and CCT is summarized in Table 1. Alkaline phosphatase in PKDs and PKDd was similar and corresponded to that seen in cultured PST. γ-Glutamyl transpeptidase was not demonstrable in any PKD cultures, although normal PST cultures were rich in this enzyme. Cytochrome oxidase staining was slight in all cultures. The most striking staining was the intense reaction of NaK-ATPase in PKDd cultures. This intensive reaction can be seen in cultured thick ascending limb of Henle's loop (TAL) from normal human tubules, but not in this intensity in PST or CCT

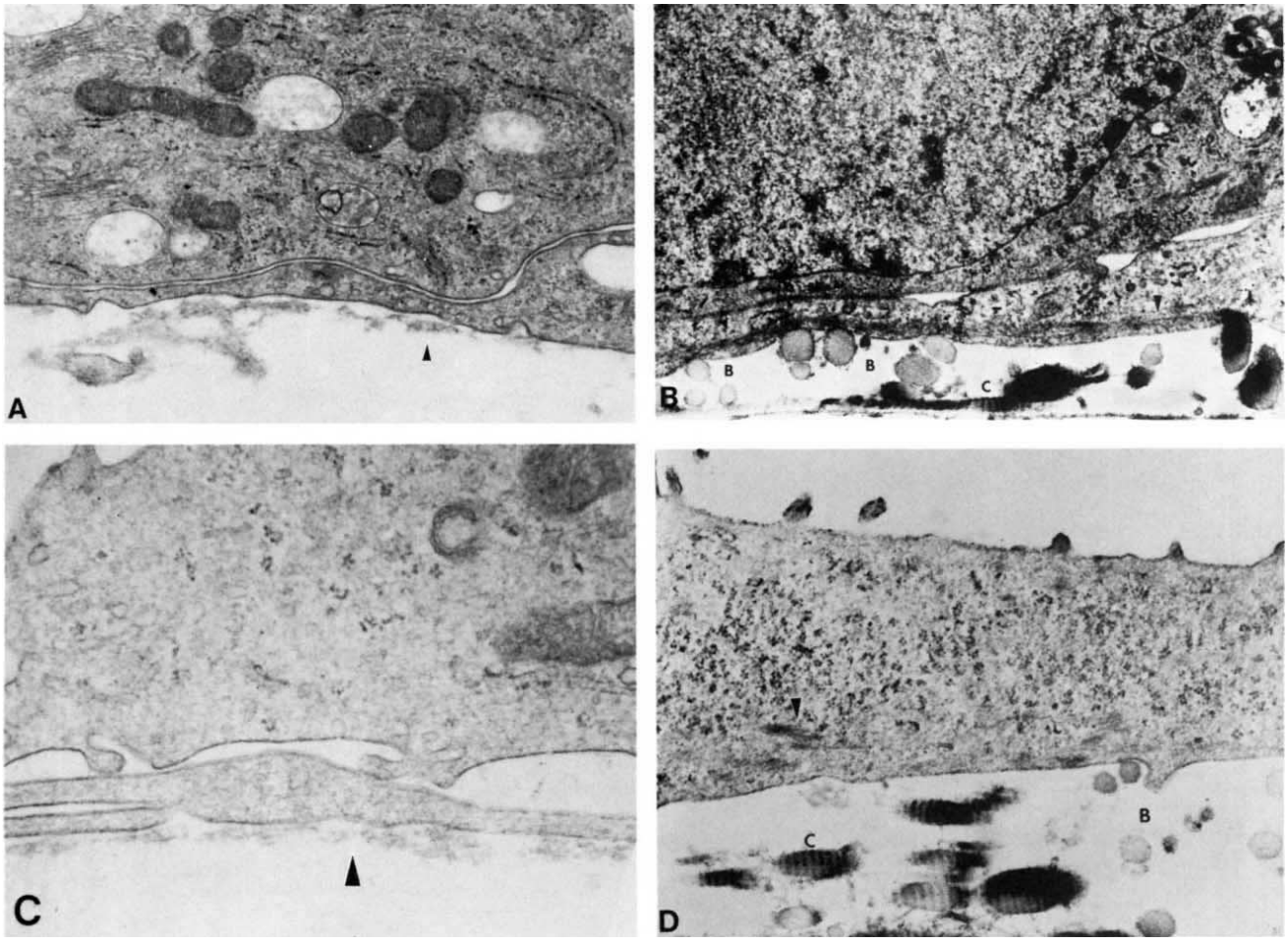


Fig. 4A. *Electron micrograph of basement membrane area of normal cultured PST cell.* A thin layer of electron dense (lamina densa–like) material is seen (↑). (×21,000) **B.** Electron micrograph of basement membrane area of polygonal PKD cell in culture showing numerous blebs (B) or spheroids of moderately electron dense material and bundles of banded collagen (C). Note the intracellular intermediate sized cytoskeletal filaments near the base of the cell. (×21,000) **C.** Electron micrograph of basement membrane area of normal CCT cell in culture showing a thin layer of electron dense (lamina densa-like) material. (×39,000) **D.** Electron micrograph of basement membrane area of elongate PKD cell in culture showing numerous blebs (B) or spheroids and many bundles of banded collagen (C). Numerous intracellular cytoskeletal filaments of intermediate size are seen (↑) near the base of the cell. (×26,500)

cultures [9]. Keratin staining was present in equal intensity in PKDs, PKDd, PST and CCT cultures.

Adenylate cyclase biochemistry

Basal adenylate cyclase activity was measurable in all cultured PKD, PST and CCT epithelia (33.4 ± 9.0 to 71.0 ± 16.0 fM cAMP/30 min/μg prot). Major differences were seen in activity stimulated by 10 U/ml parathyroid hormone. PKDs and PKDd demonstrated less responsiveness (37.3 ± 11.0 and 74.5 ± 27.0 fmoles, respectively) when compared to normal PST adenylate cyclase which was significantly stimulated (310 ± 98 fmoles, $P < 0.05$) (Fig. 7). Similarly, adenylate cyclase response to 10^{-6} M AVP was also diminished in PKDs and PKDd cultures (9.1 ± 2.0 and 16.1 ± 4.0 fmoles, respectively) in comparison to CCT cultures (510 ± 138, $P < 0.005$) (Fig. 8). Forskolin stimulated PKDs and PKDd adenylate cyclase (290 ± 93 and 507 ± 145 fmoles) was also significantly lower than in PST or CCT cultures (5597 ± 465 and 6520 ± 600 fmoles, respectively, $P < 0.005$) (Fig. 9).

Discussion

The primary culture technique of nephron segments individually isolated without the use of collagenease has been successfully applied to rabbit and human renal epithelia. Both have been shown to retain differentiated characteristics [9, 22, 23]. The present study demonstrated that human PKD epithelia can also be grown successfully applying similar microdissection, isolation, and culture techniques. These cultured cells retain the ultrastructural characteristics of cyst epithelia in vivo and express some abnormal structural and functional characteristics, suggesting the ideal applicability of this methodology for the study of the pathogenesis of human PKD.

Cultured PKD epithelia differ from normal cultured PST and CCT cells in growth pattern, cellular and extracellular matrix, ultrastructure, enzyme activities, and hormone response. The ability of PKD epithelia to grow more rapidly than normal renal epithelia in culture may suggest a fundamental difference in proliferative potential of PKD cells. This would correlate with

Table 1. Cytochemistry of cultured human normal nephron segments and polycystic kidney epithelia

Culture	Aklaline phosphatase	γ-Glutamyl transpeptidase	Cytochrome oxidase	NaK-ATPase	Keratin (LE61)
Normal PST	++	+++	++	++	++
Freshly isolated PKDs	++	±	+	+++	++
PKDs	++	−	+	++	++
Normal CCT	+	−	±*	+	++
Freshly isolated PKDd	++	−	+	+++	++
PKDd	++	−	+	+++	++

+++ strong reaction
++ moderate reaction
+ weak reaction
± very weak reaction
− no reaction
* principal cells

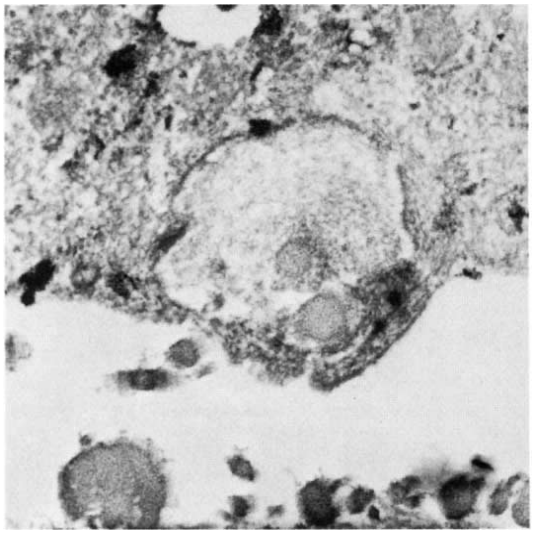


Fig. 5. *High power electron micrograph of PKD intracellular vacuole containing and apparently extruding two blebs.* The vacuole also contains finely fibrillar material. (×39,000)

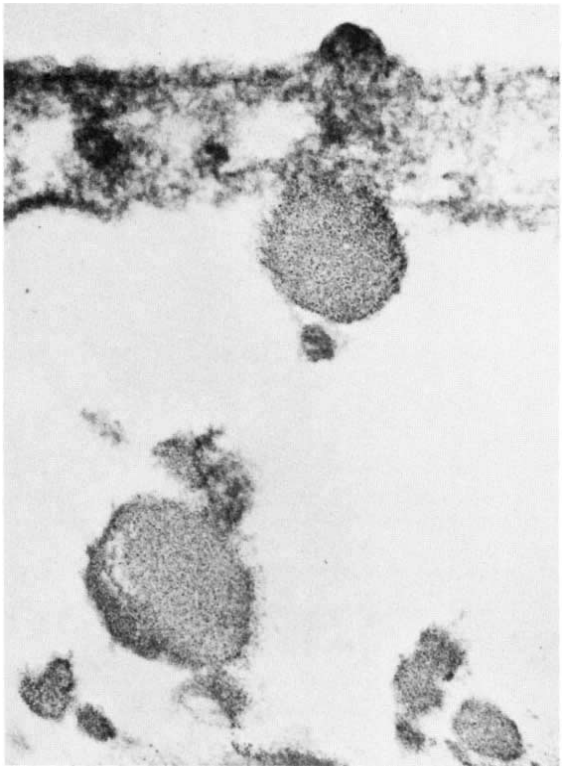


Fig. 6. *High power electron micrograph of PKD cell and blebs after ruthenium red treatment.* Note fine granular staining associated with extracellular basal blebs and with the apical cell coat. (×83,000)

the occurrence of hyperplastic polypoid lesions in intact polycystic kidneys [2], and would be consistent with the conclusions drawn from theoretical calculations invoking the need for an increased cell number to promote cyst formation [24]. Our preliminary studies show that cultured PKD cells can be subcultured, but further studies are required to determine whether they have the capacity to form a permanent cell line which would be consistent with neoplastic or pre-neoplastic potential.

Comparison of the morphology of PKD cultures suggest that these cells retain the capacity to form denuded "cyst-like" areas. These did not resemble the raised domes of "hemicysts" frequently seen in cultures of normal transporting epithelia. The majority of cells formed an intact monolayer of polygonal cells, typical of cultured epithelia, but areas were frequently seen which contained no cells and were lined by extremely elongated cells. Since both these elongated and polygonal cells were stained with LE61 antikeratin antiserum, their epithelial nature was substantiated. No fibroblastic cells were seen since the low level of FCS (3%) in the media would not support the growth of mesenchymal cells contaminating the original explanted material. Transmission electron microscopy studies also showed the presence of two cell types in PKD cultures, one somewhat flattened and one extremely flattened. This contrasted with the growth and morphology of renal epithelia from normal human kidneys which formed tightly packed homogeneous monolayers of polygonal cells.

The most striking structural abnormality seen in PKD cultures was in the ultrastructure of the basement membrane area

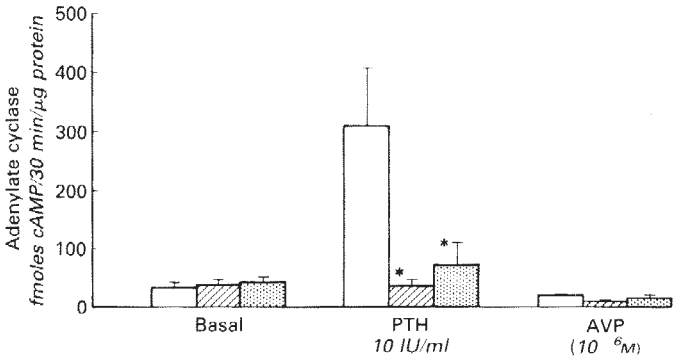


Fig. 7. *Adenylate cyclase activity in PKDs, PKDd, and PST cultures.* Symbols are: □ Normal PST; ▨ PKDs; ▩ PKDd; * $P < 0.05$.

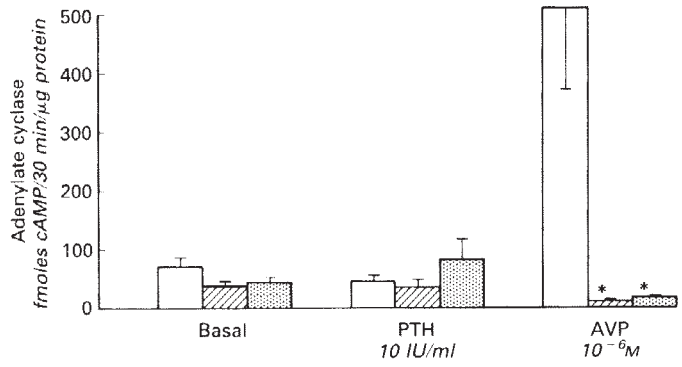


Fig. 8. *Adenylate cyclase activity in PKDs ▨, PKDd ▩, and CCT □ cultures.* $P < 0.05$.

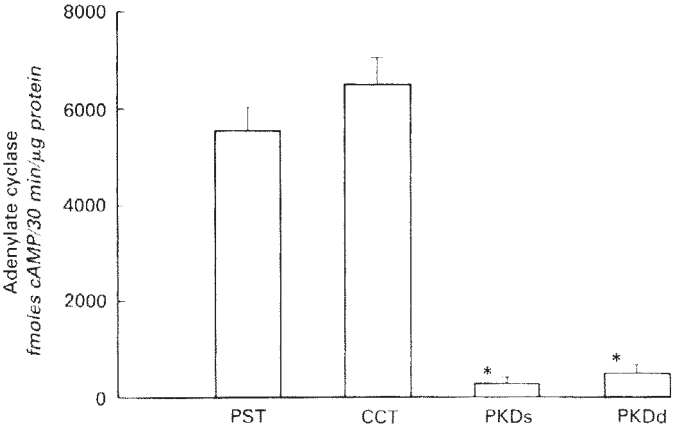


Fig. 9. *Adenylate cyclase activity response to forskolin in PST, CCT, PKDs, and PKDd cultures* (*$P < 0.005$).

Table 2. Adenylate cyclase activity* in freshly isolated and cultured PKD cysts

	Basal	PTH (10 U/ml)	AVP (10^{-6} M)	Forskolin (25 μM)
PKDs				
Fresh	30 ± 7	29 ± 12	32 ± 14	87 ± 34
Culture	37 ± 9	43 ± 15	29 ± 17	168 ± 44
PKDd				
Fresh	28 ± 13	35 ± 17	25 ± 10	104 ± 55
Culture	38 ± 9	57 ± 24	20 ± 14	269 ±74

* Activity expressed as fmole cAMP generated per 30 min per μg protein. Each value represents the mean of at least four samples.

of monolayers. Normal renal epithelia apparently produce a relatively normal basement membrane consisting of a thin electron lucent region (lamina rara) immediately adjacent to the base of the cell and a thin electron dense (lamina densa) region. This corresponds to the ultrastructural characteristics of the extracellular matrix described in normal renal tubules in intact kidneys, where it has been shown that the lamina rara contains the non-collagenous protein laminin and some proteoglycans, while the lamina densa contains type IV collagen [25, 26]. Cultured PKD cells of polygonal or flattened morphology derived from superficial and distal cysts all showed a remarkable increase in width and structure of the basement membrane area due to the presence of numerous blebs or spheroids and bundles of banded collagen similar to interstitial type I collagen. These structures were unique to the extracellular matrix of PKD epithelia. Similar blebs or spheroids were seen in intracellular vacuoles and some profiles suggested their extrusion from the cells. This suggests that PKD cells synthesize an abnormal basement membrane, and constitutes the strongest evidence to date implicating a defect in extracellular matrix as the principal lesion in PKD. This hypothesis is supported by the systemic manifestations of this disease, which include not only renal cysts but also hepatic and ovarian cysts as well as colonic diverticulae [27], berry aneurysms [5], and cardiovascular lesions [28]. In addition, a basement membrane abnormality has been suggested by previous electron microscopy studies [1–3], and preliminary biochemical results from experimental PKD demonstrating an abnormal composition [6]. Moreover, a defect in synthesis of extracellular matrix could explain the manifestation of altered cell growth such as the renal cyst polypoid lesion. Recent studies clearly demonstrate that the nature of the extracellular matrix is a critical determinant of cell growth, cell shape, polarity, and motility [29].

Previous morphological studies of intact human polycystic kidneys have shown that cysts may arise from enlargement of several areas of the nephron including proximal tubules, limbs of Henle, and collecting ducts [30]. Proximal and distal cysts have been distinguished by virtue of the pH and sodium content of the cyst fluid [1, 31]. In the present studies, the cysts were cultured from superficial and deep sites of end-stage kidneys but no differences were detected in morphology, enzymes, or hormone response between cultured PKDs and PKDd cells. However, it should be emphasized that a superficial site of origin may not necessarily correlate with a proximal cyst, and a deep origin may not necessarily correspond to a distal cyst. Enzyme activities showed that differentiated functions associated with γ-glutamyl transpeptidase activity in normal PST were absent from PKDs and PKDd cultures. The cytochrome oxidase reaction was weak and this suggested low mitochondrial content. However, a strikingly strong reaction for NaK-ATPase in PKD cells and presence of alkaline phosphatase in both PKDs and PKDd cysts and cultures suggest these cells are capable of ion transport as demonstrated in cysts in vivo [1].

Effects of parathyroid hormone and arginine vasopressin,

which stimulate adenylate cyclase activity in normal PST and CCT epithelia, respectively, did not aid in discriminating between proximal and distal origin of cysts since neither hormone elicited a stimulatory response in PKD cultures. This finding suggests a receptor defect in these cells. Forskolin, a potent diterpene stimulator of the catalytic subunit of adenylate cyclase, also showed a diminished response in PKDs and PKDd cultures, suggesting malfunction or loss of sensitivity of adenylate cyclase in these cells.

In summary, this study shows that cultured PKD cells have both structural and functional abnormalities, including an abnormal growth pattern, bizarre basement membrane ultrastructure, high NaK-ATPase, and diminished response to hormones. The chemical nature underlying the striking abnormalities in the extracellular matrix production of PKD epithelia remains to be determined although our preliminary studies with ruthenium red staining suggest a role for proteoglycans. Primary cultures of human PKD epithelia provide an important new tool for the systematic analysis of cellular and subcellular defects underlying the pathogenesis of this common disorder.

Acknowledgments

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