In Vitro Cell. Dev. Biol.—Animal 35:465–471, September 1999

1071-2690/99 $05.00+0.00

THE INFLUENCE OF CULTURE MEDIA ON EMBRYONIC RENAL COLLECTING DUCT CELL DIFFERENTIATION

KARL SCHUMACHER,[1] RAIMUND STREHL, SABINE KLOTH, MICHEL TAUC, AND WILL W. MINUTH

Department of Anatomy, University of Regensburg, Regensburg, Germany (K. S., R. S., S. K., W. W. M.) and Department of Cellular and Molecular Physiology, University of Nice, Nice, France (M. T.)

(Received 8 October 1998; accepted 2 April 1999)

SUMMARY

During kidney development the embryonic ampullar collecting duct (CD) epithelium changes its function. The capability for nephron induction is lost and the epithelium develops into a heterogeneously composed epithelium consisting of principal and intercalated cells. Part of this development can be mimicked under in vitro conditions, when embryonic collecting duct epithelia are isolated from neonatal rabbit kidneys and kept under perfusion culture. The differentiation pattern is quite different when the embryonic collecting duct epithelia are cultured in standard Iscove's modified Dulbecco's medium as compared to medium supplemented with additional NaCl. Thus, the differentiation behavior of embryonic CD epithelia is unexpectedly sensitive.

To obtain more information about how much influence the medium has on cell differentiation, we tested medium 199, basal medium Eagle, Williams' medium E, McCoys 5A medium, and Dulbecco's modified Eagle medium under serum-free conditions. The experiments show that in general, all of the tested media are suitable for culturing embryonic collecting duct epithelia. According to morphological criteria, there is no difference in morphological epithelial cell preservation. The immunohistochemical data reveal two groups of expressed antigens. Constitutively expressed antigens such as cytokeratin 19, P_{CD} 9, Na/K ATPase, and laminin are present in all cells of the epithelia independent of the culture media used. In contrast, a group of antigens detected by mab 703, mab 503, and PNA is found only in individual series. Thus, each culture medium produces epithelia with a very specific cell differentiation pattern.

Key words: kidney; development; electrolytes; perfusion culture; serum free conditions.

INTRODUCTION

Little knowledge is available about cellular biological mechanisms leading from a homogeneously to a heterogeneously composed collecting duct (CD) epithelium during kidney development. The epithelium derives from a highly specialized tissue within the embryonic CD ampulla. At this site the inducer is localized, which generates all of the nephron anlagen (28,30). Here and further downwards within the ampulla, frequent cell divisions are found, which cause the CD tubule to elongate. Beyond the neck the shaft zone starts with a sharp border where the adult and heterogeneously composed epithelium consisting of principal (P) and different kinds of intercalated (IC) cells arises (1,5,6,11).

We investigate factors affecting the transition from an embryonic tissue to an adult renal tissue with its different cell types. On one hand the functional development depends on structural elements such as the extracellular matrix (31), and on the other hand, on soluble morphogenic substances such as vitamin A derivatives (35), growth factors, or hormones (36). Further, it was found recently that not only changes in the pH or osmolarity (9,12,26), but also variations in the extracellular electrolyte composition (22,32) play an important role in the development of individual cell features.

Because of the cellular heterogeneity within the kidney, functional aspects of CD development are more easily investigated under in vitro than under in vivo conditions. These experiments presuppose that the embryonic CD cells are not kept as a simple monolayer on the bottom of a culture dish but rather, on a tissue carrier and within their specific extracellular matrix (17,20). Under static culture conditions in medium containing serum and aldosterone, a P cell type develops with amiloride-sensitive Na transport and tight characteristics (8). In contrast, under perfusion culture and in serum-free medium, aldosterone triggers the development not only of P but also of β-type IC cell features by increasing the amount of peanut lectin (PNA)-binding cells (19). In addition, treatment of the CD epithelia with culture medium supplemented with additional NaCl evokes in the embryonic epithelia the primary appearance of further P and IC cell features (22,32).

Regarding cell differentiation, we did not realize before how sensitive the embryonic CD epithelium is to the influence of the environment within the medium. To obtain further information about this effect, we tested the influence of six different culture media on cell differentiation under serum-free conditions in the embryonic CD epithelium. We analyzed whether all culture media trigger the expression of the same differentiation profile, or whether different programs can be activated in the embryonic CD epithelium.

[1]To whom correspondence should be addressed at Department of Anatomy, Universitätsstrasse 31, University of Regensburg, D-93053 Regensburg, Germany.

Materials and Methods

Tissue isolation and generation of an embryonic collecting duct epithelium. Cortical explants from the kidneys of newborn New Zealand rabbits were isolated according to methods described earlier (17). The explants consisted of a thin piece of capsula fibrosa with adherent collecting duct ampullae, S-shaped bodies, and nephrogenic blastema. Then the tissue was mounted in sterile carriers (Fig. 1 *A*; Minucells and Minutissue, Bad Abbach, Germany), which were placed in 24-well culture plates (Greiner, Nürtingen, Germany). During the culture of these explants in Iscove's modified Dulbecco's medium (IMDM; GIBCO BRL-Life Technologies, Eggenstein, Germany) and 10% fetal bovine serum (Boehringer, Mannheim, Germany) an outgrowth of cells from the collecting duct ampullae was observed. Within 24 h after the initiation of culture, the entire surface of the explant, 6 mm in diameter, was covered by a polarized collecting duct epithelium. Culture for the first d was carried out in a tissue incubator (Heraeus, Hanau, Germany) at 37° C in a humidified atmosphere containing 5% CO_2 and 95% air.

Perfusion culture of embryonic collecting duct epithelia. Twenty-four h after the initiation of culture the epithelia on the tissue carriers (Fig. 1 *A*) were transferred to a perfusion culture container (Fig. 1 *B*; Minucells and Minutissue, Bad Abbach, Germany) to create improved culture conditions (20). Fresh medium was continuously perfused for an additional 13 d at a rate of 1 ml/h with an IPC N8 peristaltic pump (Ismatec, Wertheim, Germany) (Fig. 1 *C*). To maintain a constant temperature of 37° C, the container was placed on a thermo plate (Medax, Kiel, Germany) and covered by a transparent lid. For each experimental series, three perfusion culture experiments with four epithelia each were performed so that 12 epithelia were examined per series of media. A total of 72 epithelia were kept in perfusion culture for the following experiments.

Culture media. Six different media were used to keep the embryonic collecting duct epithelia in perfusion culture. All of the media were purchased from GIBCO BRL-Life Technologies, Eggenstein, Germany. Iscove's modified Dulbecco's medium (IMDM; Nr. 21980) (2) served as the known standard. Medium 199 (M 199; Nr. 31153) (25), basal medium Eagle (BME; Nr. 42300) (4), Williams' medium E (WME; Nr. 32551) (37), McCoys 5 A medium (MCM; Nr. 22330) (15) and Dulbecco's modified Eagle medium (DMEM; Nr. 20170) (2) were used for the experimental series.

Additives of the culture media. None of the media contained serum or growth factors during perfusion culture. Aldosterone (1×10^{-7} *M*; Sigma-Aldrich-Chemie, Deisenhofen, Germany) and 1% antibiotic-antimycotic solution (GIBCO) was added to all of the perfusion culture media. Furthermore, depending on sodium bicarbonate concentration, up to 100 mmol of *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES) (GIBCO) per l was added to maintain a constant pH of 7.4 in perfusion culture under laboratory air atmosphere (0.3% CO_2).

Physiological parameters of the culture media. Culture parameters such as pH, pCO_2, pO_2, lactate, osmolarity, or electrolyte contents such as Na^+, K^+, Cl^-, and Ca^{2+} were determined in an undiluted 200-µl sample by a Stat Profile 9 Plus analyzer according to the manufacturer's instructions (Nova Biomedical, Rödermark, Germany). Solutions with defined electrolyte concentrations served as controls. The electrolyte composition of the individual culture media is listed in Table 1. The media contained sodium bicarbonate in the following amounts per dl: IMDM, 3024 mg; M199, 1250 mg; BME, 2200 mg; WME, 2200 mg; MCM, 2200 mg and DMEM 3700 mg. In all perfusion culture experiments, O_2 content in the media was above 168 mm Hg and CO_2 content was above 9 mm Hg.

Histochemical markers. Primary appearance of CD cell features was determined by immunohistochemistry and monoclonal antibodies (mabs) against CD antigens. The following antibodies were used to identify CD cell features: The anti-cytokeratin 19 antibody recognizes a 40-kDa polypeptide in all collecting duct cells in the kidney and was kindly provided by Prof. Dr. R. Moll, Marburg, Germany (24). The antibody was used in 1:10 dilution. Mab P_{19} 9 detects 32- and 39-kDa proteins on all of the collecting duct cells of the neonatal kidney and was generated in our laboratory (14). A mab-detecting Na/K-ATPase binds to a cytosolic epitope of the α-subunit of CD cells (16). The mab was developed by Dr. Douglas M. Fambrough and obtained from the Development Studies Hydroma Bank (University of Iowa, Department of Biological Sciences, Iowa City, IA 52242, under contract NO1-HD-7-3263 from the NICHD). The anti-laminin antibody (Boehringer Mannheim, Germany) reacts with a 900-kDa protein in the basement membrane of all epithelia of the rabbit kidney. A 1:50 diluted antibody solution was applied. Mab 703 and mab 503 were generated in the laboratory of Dr. M. Taue, Nice, France and used in dilution of 1:100 (34). Mab 703 detects a 70-kDa protein of P-Cells, whereas mab 503 recognizes a 31-kDa protein of IC cells of the adult collecting duct cells. Mab P_{19} Amp was generated in our laboratory and detects 32- and 39-kDa proteins in the basal region of ampullar collecting duct epithelium (33). Mab MIB 1 binds to the protein Ki 67 of cells in the mitotic cycle (7). It was used in a dilution of 1:50. The antibody was a kind gift from Prof. Dr. G. Gerdes, Borstel, Germany. If not mentioned otherwise,

FIG. 1. Perfusion culture of a renal collecting duct epithelium. *A*, The renal explant 6 mm in diameter is mounted in a tissue carrier. Within the first 24 h of culture a collecting duct epithelium (E) is developed, which covers the complete upper surface of the explant. *B*, Six carriers can be placed in a perfusion culture container. *C*, A peristaltic pump transports the medium (flow rate 1 ml/h) and a thermo plate ensures a constant temperature of 37° C during the 14-d culture period.

TABLE 1

ELECTROLYTES, GLUCOSE AND OSMOLARITY IN CULTURE MEDIA USED TO TEST THEIR EFFECTS ON EMBRYONIC RENAL DUCT CELL DIFFERENTIATION

Parameter	Concentration	Iscove's modified Dulbecco's medium	Medium 199	Basal medium Eagle	Williams' medium E	McCoys 5A medium	Dulbecco's modified Eagle medium
Na^+	(mmol/l)	117.8	138.9	146.2	144.4	142.6	157.7
Cl^-	(mmol/l)	81.5	125.3	111.2	117.9	106.4	116.9
K^+	(mmol/l)	3.9	5.1	4.8	4.8	4.8	4.8
Ca^{++}	(mmol/l)	1.1	1.5	1.4	1.4	0.5	1.3
Glucose	(mg/dl)	418.3	99.6	94.2	186.3	270.3	382.4
Osmolarity	(mOsm)	250.0	270.0	286.0	288.1	289.6	323.8

[a]The mean values of at least six meansurements are given.

the antibodies were used as undiluted culture supernatants. Later on, peanut agglutinin (PNA) (Vector, Burlingame, VT) was used, which binds to terminal D-galactosyl residues of glycoproteins of β-type IC-cells of the rabbit collecting duct (29). PNA was applied at a dilution of 1:2000.

Immunohistochemistry. For microscopical examinatiou of cultured epithelia and for immunohistochemical detection of collecting duct proteins 8-μm-thick cryosections were prepared with a Cryostat HM 500 (Microm, Walldorf, Germany). For histological control, sections were stained with toluidine blue solution. Immunolabeling was started by fixing the cryosections for 10 min in ice-cold ethanol as described earlier (13). After several rinses with phosphate-buffered saline (PBS), pH 7.2, the sections were incubated with a blocking solution (PBS) containing 10% horse serum and 1% bovine serum albumin (BSA) for 30 min. The primary antibodies were incubated for 1.5 h. After several rinses with PBS and 1% BSA, the sections were treated for 45 min with a donkey-anti-mouse-IgG-fluorescein-isothiocyanate (FITC)-conjugated secondary antibody (Jackson Immunoresearch Laboratories, West Grove, PA) diluted 1:200 in blocking buffer. After being rinsed several times in PBS and 1% BSA, the specimens were coincubated for 45 min with a PNA–rhodamine conjugate (Vector) diluted 1:2000 in PBS to detect β-type IC cell features (29). After several washes with PBS, the sections were embedded in FITC guard (Testoc, Chicago, IL) and examined with an Axiovert 35 microscope (Zeiss, Oberkochen, Germany).

From each epithelium, at least 100 cryosections were analyzed. About 150 cells were scored within the epithelium for each cryosection. To determine the number of immunopositive cells in the cultured epithelia a double labeling procedure was applied. The epithelia were first labeled with the nuclear marker propidium iodide (4 μg/ml in PBS; Sigma-Aldrich-Chemie, Deisenhofen, Germany; data not shown) and then with a further cellular marker. By this method the amount of mab-labeled and unlabeled cells within the epithelium could easily he determined. The mean of immunopositive cells within the epithelium is given in the text.

Results

We used embryonic CD epithelia to investigate the mechanisms involved in the development and preservation of individual cell differentiation. A prerequisite for reaching a high degree of cellular differentiation under in vitro conditions is that the epithelia are kept in an environment which is optimized as far as possible (16,18,20,21). The embryonic tissue grows in a carrier within its organ-specific extracellular matrix (Fig. 1 *A*), in a specific culture container (Fig. 1 *B*) and with a permanent exchange of fresh medium (Fig. 1 *C*). To test the influence of culture media on differentiation in the following experimental series, the embryonic CD epithelia were exposed to Iscove's modified Eagle medium (IMDM) with low Na content, medium 199 (M 199), basal medium Eagle (BME), Williams' medium E (WME), and McCoys 5 A medium (MCM), all with medium Na content, and Dulbecco's modified Eagle medium (DMEM) with high Na content (Table 1).

Independent of the culture media used in all of the experimental series, a renal CD epithelium became established after 14 d of perfusion culture and was harvested. Among the different experimental series, differences in cell quality could not be seen according to pure morphological criteria. All of the epithelia appeared well preserved and showed a clear polarization. The luminal side faced the culture medium, while the basal area rested on a basement membrane and the kidney-specific extracellular matrix. Since by the administration of IMDM, M 199, BME, WME, MCM and DMEM an excellently preserved epithelium could be harvested, all of the media appear to be suitable in principle for future cell culture protocols.

During development, maturing CD epithelium in the kidney undergoes a mitotic and a subsequent differentiation phase without cell proliferation (23). All of the culture media used were originally made to keep proliferating cells in culture. Thus, it is not known whether the above-mentioned media are suitable for the maintenance of postmitotic tissue. When we analyzed proliferation activity in the cultured embryonic CD epithelium, we observed multiple dividing cells after Day 1, whereas between Day 2 and 5 the number decreased. After Day 7 cell divisions were no longer visible (data not shown) (23). We found without exception that in all of the culture media tested so far, the CD epithelium in culture reached a postmitotic state within 14 d as observed in the adult kidney (Fig. 2 *A–F*).

The cultured CD epithelia derive from embryonic tissue, which undergoes a maturation process under in vitro conditions. It is thus important to control the transition from the embryonic to the mature state. The embryonic CD marker mab P_{CD} Ampl showed a clear immunohistochemical reaction at the basal part of the CD ampulla in neonatal rabbit kidney (33). In contrast, no reaction was found in the maturing or matured CD epithelium of the kidney. No immunohistochemical reaction with mab P_{CD} Ampl was found on CD epithelia cultured with any of the six different media after 14 d (Fig. 2 *G–L*). In this respect the epithelia lost their embryonic character in all of the series.

We tested the primary appearance of typical structural and functional features during CD development by a set of monoclonal antibodies after culture of the epithelia in different media (Fig. 3–6; Table 2). Surprisingly, we found two groups of reaction pattern. The one group showed constitutive antigen expression. Independent of the culture medium used, all of the embryonic cells in the epithelia showed primary upregulation of CD features. The second group comprised facultative antigens, whose expression depended on the culture media used.

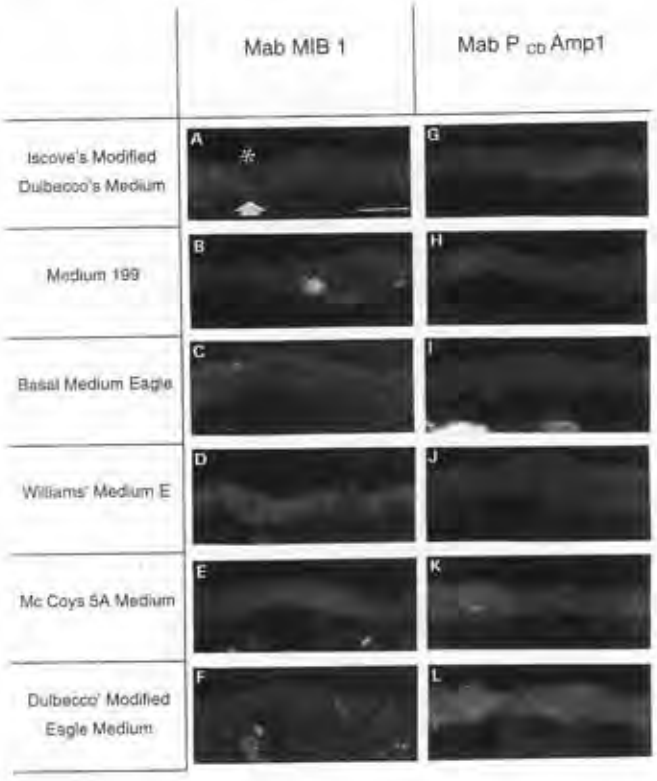

FIG. 2. Downregulation of mitotic activity detected by mab Mib 1 (*A–F*) and embryonic characteristic detected by mab P $_{CD}$ Amp1 (*G–L*) after 14 d of culture. The epithelia were cultured under serum-free conditions and without growth factors. *Bar*, 20 μm; *asterisk*, luminal side; *arrow*, basal portion of the epithelium.

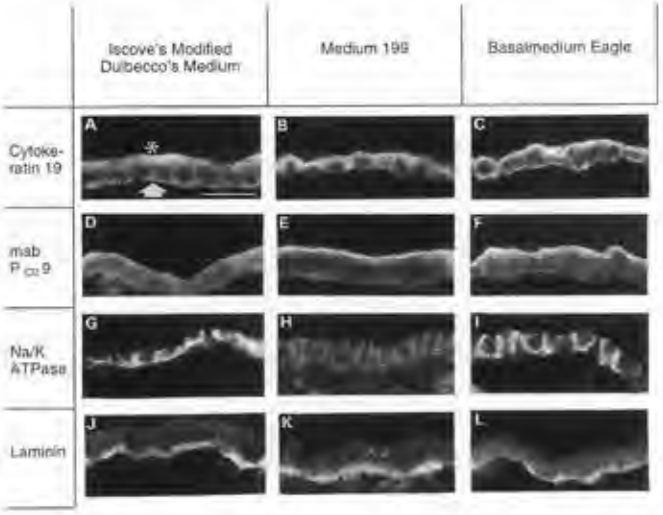

FIG. 3. Constitutive development of cell features in CD epithelia cultured with IMDM (*A–J*), M 199 (*B–K*) and BME (*C–L*) after 14 d. Typical features such as cytokeratin 19 (*A–C*), P_{CD} 9 (*D–F*), Na/K ATPase (*G–I*) and laminin (*J–L*) were found in all of the CD cells and independently of the culture media used. *Bar*, 20 μm; *asterisk*, luminal side; *arrow*, basal portion of the epithelium.

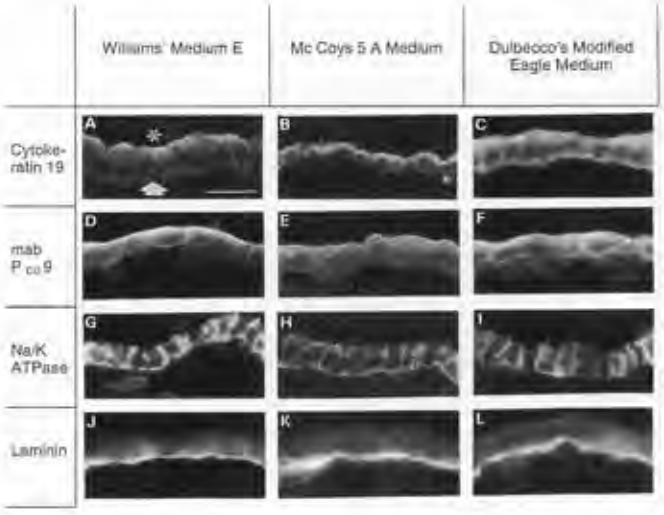

FIG. 4. Constitutive development of cell features in CD epithelia cultured with WME (*A–J*), MCM (*B–K*) and DMEM (*C–L*) after 14 d. Typical features such as cytokeratin 19 (*A–C*), P_{CD} 9 (*D–F*), Na/K ATPase (*G–I*) and laminin (*J–L*) were present in all of the CD cells and independently of the culture media used. *Bar*, 20 μm; *asterisk*, luminal side; *arrow*, basal portion of the epithelium.

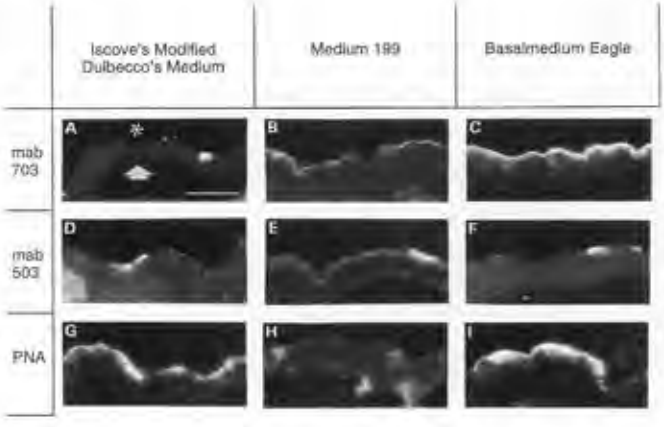

FIG. 5. Facultative development of cell features in CD epithelia cultured with IMDM (*A–G*), M 199 (*B–H*) and BME (*C–I*). Ninety to 100% mab 703-positive cells were found only after culture in BME (*C*). Eighty to 90% PNA-positive cells were detected only in series with IMDM (*G*) and BME (*I*). *Bar*, 20 μm; *asterisk*, luminal side; *arrow*, basal portion of the epithelium.

When markers against constitutively expressed antigens of CD cells such as cytokeratin 19 (24) (Fig. 3 *A–C*, 4 *A–C*), P_{CD} 9 (14) (Fig. 3 *D–F*, 4 *D–F*), Na/K ATPase (16) (Fig. 3 *G–I*, 4 *G–I*) and laminin (3) (Fig. 3 *J–L*, 4 *J–L*) were tested, in all of the cases an intensive immunohistochemical reaction was found in all epithelial CD cells. All cells were immunopositive although electrolyte composition of the media differed (Table 1), the immunohistochemical pattern (Fig. 3–4) for the above-mentioned antigens did not vary.

In contrast, labeling of the CD epithelia with newly developed antibodies such as mab 703-detecting P cells and mab 503-labeling IC cells in the adult kidney (34) demonstrated that an individual

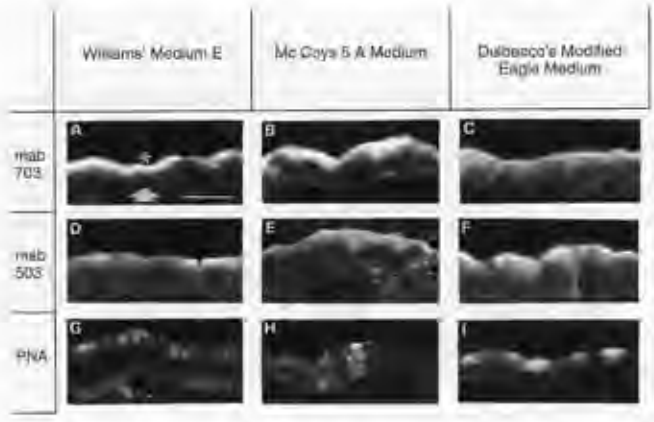

FIG. 6. Facultative development of cell features in CD epithelia cultured with WME (*A–G*), MCM (*B–H*) and DMEM (*C–I*). Ninety to 100% mab 703-positive cells were found only in series with WME (*A*), MCM (*B*) and DMEM (*C*). Up to 70% immunopositive cells for mab 503 were present after culture in WME (*D*) and MCM (*E*), and 70 to 90% after culture in DMEM (*F*). In contrast, PNA binding was found in only 10 to 20% after culture in WME (*G*) and in 5 to 10% after application of MCM (*H*). In DMEM, 70 to 80% positive cells were detected (*I*). *Bar*, 20 μm; *asterisk*, luminal side; *arrow*, basal portion of the epithelium.

pattern of cell differentiation was obtained, depending on the culture medium used (Table 2; Fig. 5–6). Culture of CD epithelium in IMDM (Fig. 5 *A*) showed binding on only 5 to 10% of the epithelial cells with mab 703. With M 199 (Fig. 5 *B*) 30 to 40% mab 703-positive cells were present. In contrast, application of BME (Fig 5 *C*), WME (Fig. 6 *A*), MCM (Fig. 6 *B*) and DMEM (Fig. 6 *C*) revealed 90 to 100% mab 703-positive cells in the cultured CD epithelium.

We investigated facultative protein expression by labeling the epithelia with mab 503 (Fig. 5–6). Only 5 to 10% of the CD cells were labeled in series with IMDM (Fig. 5 *D*), M 199 (Fig. 5 *E*) and BME (Fig. 5 *F*). In contrast, up to 70% of cells were immunopositive when the CD epithelia were cultured in WME (Fig. 6 *D*) and MCM (Fig. 6 *E*). Between 70 and 90% of the cells were mab 503-positive when the epithelia were cultured in DMEM (Fig. 6 *F*).

PNA is a marker for mature β-type IC cells in the rabbit kidney (29). When we applied this marker to the cultured CD epithelia, between 70 and 90% of the CD cells showed PNA binding in cultures in IMDM (Fig. 5 *G*), BME (Fig. 5 *I*) and DMEM (Fig. 6 *I*). In contrast, only 5 to 20% positive cells were labeled in cultures with M 199 (Fig. 5 *H*), WME (Fig. 6 *G*) and MCM (Fig. 6 *H*).

DISCUSSION

We have tried to illuminate a new mechanism which triggers renal CD cell differentiation by the medium. Earlier experiments showed that addition of NaCl and Na gluconate to IMDM changes the differentiation profile as compared to the standard culture protocol (22,32). Not by but simply by adding NaCl during serum-free culture rather than a hormone or a growth factor, we changed the differentiation profile. In the present study, we wanted to determine whether differentiation changed in only the IMDM treatment or whether the CD epithelia are in general sensitive to the composition of different culture media. We thus kept embryonic CD epithelia 13 d in perfusion culture in parallel series with IMDM, M 199, BME, WME, MCM, and DMEM under serum-free conditions (Fig. 2–5). The media were chosen because of their low (IMDM), medium (M 199, BME, WME, MCM) and high (DMEM) Na content. We are aware that these media differ not only in their electrolyte composition but also in amino acid content and other nutritional factors. Without the influence of serum or growth factors, we recorded the differentiation profile of embryonic CD epithelia cultured in these six different media.

Our first important result is that according to morphological criteria in all six experimental series with different culture media, polarized CD epithelia developed. Labeling of the epithelia with mab MIB 1 to register mitotic activity demonstrated that proliferation completely ceased after 14 d of culture (Fig. 2 *A–F*). At Day 14, none of the CD cells showed a fluorescent signal, clearly demonstrating that the CD cells cultured on an organ-specific matrix reached a postmitotic state. This parallels the conditions within the kidney. In the neonatal CD ampulla, numerous cells with mab MIB 1 were labeled, whereas no label was found in the mature parts of the CD (23).

Our second important result was that the immunohistochemical differentiation profiles depend on the culture medium used in the different experiments. Protein expression is triggered by a facultative and a constitutive mechanism. Constitutive molecules are cytokeratin 19, P_{CD} 9, Na/K ATPase, and laminin. All of these proteins were present in all of the experimental series (Fig. 3,4). The second group of markers represents the facultatively expressed molecules, which are tightly regulated depending of the culture medium. This group comprises antigens detected by mab 703, mab 503, and PNA and can be observed in only individual series (Table 2; Fig. 5,6). IMDM as the standard medium generates the smallest degree of typical cell differentiation (Fig. 5 *A,D*). All the other media generated differentiation to a much greater degree. For example, 40 to 100% of cells are labeled by mab 703 or mab 503 after treatment with WME (Fig. 6 *A,D*), MCM (Fig. 6 *B,E*) and DMEM (Fig. 6 *C,F*).

TABLE 2

FACULTATIVE DEVELOPMENT OF CELL FEATURES IN CULTURED CD EPITHELIA[a]

Cell feature	Iscove's modified Dulbecco's medium	Medium 199	Basal medium Eagle	Williams' medium E	McCoys 5a medium	Dulbecco's modified Eagle medium
703	5 to 10%	30 to 40%	90 to 100%	90 to 100%	90 to 100%	90 to 100%
503	5 to 10%	5 to 10%	5 to 10%	40 to 60%	30 to 70%	70 to 90%
PNA-binding	80 to 90%	5 to 10%	80 to 90%	10 to 20%	5 to 10%	70 to 80%

[a] Immunopositive cells in percent.

From culture experiments it is known that variation in osmolarity causes changes in cytodifferentiation (10,27). It appears likely that the variation in osmolarity in our culture media was responsible for the change in cell differentiation of the CD epithelia. However, we observed that culture of the epithelia in media with nearly the same osmolarity such as BME (286 mOsm; Fig. 5 *C,F,I*; Tables 1,2), WME (288 mOsm; Fig. 6 *A,D,G*; Tables 1,2) and MCM (289 mOsm; Fig. 6 *B,E,H*; Tables 1,2) did not show the same profile of cytodifferentiation as expected. In contrast, a quite different and individual pattern was observed. This result demonstrates that environmental factors other than osmolarity influence the CD cell differentiation profile.

Further candidates for triggering differentiation are the electrolytes, since administration of NaCl or Na gluconate to IMDM changes the differentiation profile of CD epithelia (22,32). Our culture media showed a low (IMDM), medium (BME, WME, MCM), or a high (DMEM) Na concentration (Table 1). For example, the lowest Na concentration of 117 mmol/l was found in IMDM, whereas the highest concentration of 156 mmol/l Na was found in DMEM. We found that the mab 703 binding on CD cells correlated with the Na content in the different culture media (low Na, low number of labeled cells; high Na, high number of labeled cells). In contrast, mab 503 binding does not correlate with increasing Na concentrations (Table 2, Fig. 3–6). In addition, the increase of PNA-positive cells does not seem to correspond to the increasing Na concentrations in the different media. It was shown in earlier studies that PNA binding could be drastically increased by addition of aldosterone to IMDM (19). Obviously, the addition of aldosterone has this effect also in BME and DMEM (Fig. 5 *G,I*, 6 *I*), but not at this intensity in M 199, WME, and MCM (Fig. 5 *H*, 6 *G*, 6 *H*). Thus, each culture medium produces its own very specific differentiation profile.

Acknowledgments

This investigation was supported by the Deutsche Forschungsgemeinschaft (Mi 331/4-2). The technical assistance of Mrs. Elfriede Eckert is gratefully acknowledged.

References

1. Aigner, J.; Kloth, S.; Minuth, W. W. Transitional differentiation patterns of principal and intercalated cells during renal collecting duct development. Epithelial Cell Biol. 4:121–130; 1995.
2. Dulbecco, R.; Freeman, G. Iscove's modified Eagle medium. Virology 8:396; 1959.
3. Durbeej, M.; Ekblom, P. Dystroglycan and laminins: glycoconjugates involved in branching epithelial morphogenesis. Exp. Lung Res. 23:109–118; 1997.
4. Eagle, H. Basal medium eagle. Proc. Soc. Exp. Biol. Med. 89:362; 1965.
5. Emmons, C.; Kurtz, I. Functional characterization of three intercalated cell subtypes in the rabbit outer cortical collecting duct. J. Clin. Invest. 93:417–423; 1994.
6. Evan, A. P.; Satlin, L. M.; Gattone, V. H.; Connors, B.; Schwartz, G. J. Postnatal maturation of rabbit renal collecting duct. II. Morphological observations. Am. J. Physiol. 261:F91–107; 1991.
7. Gerdes, J.; Li, L.; Schlueter, C.; Duchrow, M.; Wohlenberg, C.; Gerlach, C. Immunobiochemical and molecular biologic characterization of the cell proliferation-associated nuclear antigen that is defined by monoclonal antibody Ki-67. Am. J. Pathol. 138:867–873; 1991.
8. Gross, P.; Minuth, W. W.; Kriz, W.; Frömter, E. Electric properties of renal collecting duct principal cell epithelium. Pflügers Arch. 406:380–386; 1986.
9. Hayashi, M.; Iyori, M.; Yamaji, Y.; Saruta, G. Effects of in vivo and in vitro alkali treatment of intracellular pH regulation of OMCD cells. Am. J. Physiol. 265:F729–735; 1993.
10. Isabelle, M. E.; Githens, S.; Moses R. L., Bartell, C. K. Culture of rat renal medullary tissue in media made hyperosmotic with NaCl and urea. J. Exp. Zool. 269:308–318; 1994.
11. Kim, J.; Tisher, C. C.; Madsen, K. M. Differentiation of intercalated cells in developing rat kidney: an immunohistochemical study. Am. J. Physiol. 266:F977–990; 1994.
12. Kleinmann, J. G.; Tipnis, P.; Pscheidt, P. H(+)-K(+)-ATPase of rat inner medullary collecting duct in primary culture. Am. J. Physiol. 265:F698–704; 1993.
13. Kloth, S.; Aigner, J.; Brandt, E.; Moll, R.; Minuth, W. W. Histochemical markers reveal an unexpected heterogenous composition of the renal embryonic collecting duct epithelium. Kidney Int. 44:527–536; 1993.
14. Kloth, S.; Gmeiner, T.; Aigner, J.; Jennings, M. L.; Röckl, W.; Minuth, W. W. Transitional stages in the development of the rabbit renal collecting duct. Differentiation 63:21–32; 1998.
15. McCoy, T. A.; Maxwell, M.; Kruse, P. F. McCoys 5A medium. Proc. Exp. Biol. Med. 100:115; 1959.
16. Minuth, W. W.; Gross, P.; Gilbert, P.; Kashgarian, M. Expression of the α-subunit of Na/K-ATPase in renal collecting duct epithelium during development. Kidney Int. 31:1104–1112; 1987.
17. Minuth, W. W. Neonatal rabbit kidney cortex in culture as tool for the study of collecting duct formation and nephron differentiation. Differentiation 36:12–22; 1987.
18. Minuth, W. W.; Dermietzel, R.; Kloth, S.; Hennerkes, B. A new method culturing renal cells under permanent superfusion and producing a luminal-basal medium gradient. Kidney Int. 41:215–219; 1992.
19. Minuth, W. W.; Fietzek, W.; Kloth, S.; Stöckl, G.; Aigner, J.; Röckl, W.; Kubitza, M.; Dermietzel, R. Aldosterone modulates the development of PNA binding cell isoforms within renal collecting duct epithelium. Kidney Int. 44:337–344; 1993.
20. Minuth, W. W.; Aigner, J.; Kloth, S.; Steiner, P.; Tauc, M.; Jennings, M. L. Culture of embryonic renal collecting duct epithelium in a gradient container. Pediatr. Nephrol. 11:140–147; 1997.
21. Minuth, W. W.; Aigner, J.; Kubat, B.; Kloth, S. Improved differentiation of renal tubular epithelium in vitro: potential for tissue engineering. Exp. Nephrol. 5:10–17; 1997.
22. Minuth, W. W.; Steiner, P.; Strehl, R.; Kloth, S.; Tauc, M. Electrolyte environment modulates differentiation in embryonic renal collecting duct epithelia. Exp. Nephrol. 5:414–422; 1997.
23. Minuth, W. W.; Steiner, P.; Strehl, R.; Schumacher, K.; de Vries, U.; Kloth, S. Modulation of cell differentiation in perfusion culture. Exp. Nephrol.: in press.
24. Moll, R.; Franke, W.; Schiller, D. The catalog of human cytokeratins: patterns of expression in normal epithelia, tumors and cultured cells. Cell 31:11–24; 1982.
25. Morgan, I.; Morton, P.; Parker, G. Medium 199. Proc. Soc. Exp. Biol. Med. 73:1; 1950.
26. Obermüller, N.; Kränzlin, R.; Verma, R.; Gretz, N.; Kriz, W.; Witzgall, R. Renal osmotic stress-induced cotransporter: expression in the newborn, adult and postischemic rat kidney. Kidney Int. 52:1584–1592; 1997.
27. Ohtaka, A.; Muto, S.; Nemoto, J.; Kawakami, K.; Nagano, K.; Asano, Y. Hyperosmolality stimulates Na-K-ATPase gene expression in inner medullary collecting duct cells. Am. J. Physiol. 270:F728–738; 1996.
28. Sariola, H.; Sainio, K. The tip-top branching ureter. Curr. Opin. Cell Biol. 9:877–884; 1997.
29. Satlin, L. M.; Matsumoto, T.; Schwartz, G. J. Postnatal maturation of rabbit renal collecting duct. III. Peanut lectin binding intercalated cells. Am. J. Physiol. 262:F199–208; 1992.
30. Saxén, L. Organogenesis of the kidney. In: Barlow, P. W.; Green, P. B.; Wylie, C. C., ed. Developmental and cell biology series. Vol. 19. Cambridge: Cambridge University Press; 1987:13.
31. Sorokin, L.; Ekblom, P. Development of tubular and glomerular cells of the kidney. Kidney Int. 41:657–664; 1992.
32. Steiner, P.; Strehl, R.; Kloth, S.; Tauc, M.; Minuth, W. W. In vitro development and preservation of specific features of collecting duct epithelial cells from embryonic rabbit kidney are regulated by the electrolyte environment. Differentiation 62:193–202; 1997.

33. Strehl, R.; Kloth, S.; Aigner, J.; Steiner, P.; Minuth, W. W. P_{CD} Ampl, a new antigen at the interface of the embryonic collecting duct epithelium and the nephrogenic mesenchyme. Kidney Int. 52:1469–1477; 1997.
34. Tauc, M.; Koechlin, N.; Jamous, M.; Ouaghi, E. M.; Gastineau, M.; Le Moal, C.; Poujeol, P. Principal and intercalated cells in primary cultures of rabbit renal collecting tubules revealed by monoclonal antibodies. Biol. Cell 76:55–65; 1992.
35. Vilar, J.; Gilber, T.; Moreau, E.; Merlet-Bénichou, C. Metanephros organogenesis is highly stimulated by vitamin A derivatives in organ culture. Kidney Int. 49:1478–1487; 1996.
36. Weller, A.; Sorokin, L.; Illgen, E. M.; Ekblom, P. Development and growth of mouse embryonic kidney in organ culture and modulation of development by soluble growth factors. Dev. Biol. 144:248–261; 1991.
37. Williams, G. M.; Gunn, J. M. Long-term cell culture of adult rat liver epithelial cells. Exp. Cell Res. 89:139; 1974.