Original Paper

Experimental Nephrology

Exp Nephrol 1997;5:414–422

Received: January 13, 1997
Accepted: April 21, 1997

Electrolyte Environment Modulates Differentiation in Embryonic Renal Collecting Duct Epithelia

Will W. Minuth[a]
Pat Steiner[a]
Raimund Strehl[a]
Sabine Kloth[a]
Michel Tauc[b]

[a] Department of Anatomy, University of Regensburg, Germany;
[b] Department of Cellular and Molecular Physiology, University of Nice, France

Abstract

The influence of electrolytes on the development of renal principal and intercalated collecting duct cells is unknown. Consequently embryonic collecting duct epithelia were exposed to different electrolyte concentrations, and their degree of differentiation was registered by immunohistochemical methods. Embryonic collecting duct epithelia were isolated from neonatal rabbit kidneys and placed on tissue carriers. The apical urine and the basal serum compartments were simulated in a gradient culture container. The two sides of the epithelium were each constantly superfused with medium for 13 days. In controls the medium on both apical and basal side was standard Iscove's modified Dulbecco's Medium (IMDM) with 112 mmol/l Na^+ and 85 mmol/l Cl^-. In experimental series the NaCl concentration at the basal side of the epithelium was increased up to 137 mmol/l Na^+ and 99 mmol/l Cl^- as found in the serum of neonatal rabbits. Light microscopy revealed morphologically faultless epithelia following gradient perfusion culture in standard and NaCl-adapted IMDM. The development of principal and intercalated cell features was monitored with the monoclonal antibodies 703, 503, $P_{CD}9$, and peanut lectin. Cells immunopositive for monoclonal antibody 703, for example, increased from less than 10% in controls to more than 80% in NaCl-adapted IMDM. It is a new finding that the development of collecting duct cell features is influenced by the extracellular electrolyte environment.

Key Words
Kidney
Collecting duct
Development, renal collecting duct
Electrolytes
Gradient perfusion culture

Introduction

We were interested in the development of principal (P) and intercalated (IC) cell features within the collecting duct (CD) of the neonatal rabbit kidney. The different cell types arise from the embryonic CD ampulla which consists of three different functional zones [1]. The ampullar tip is embedded in the nephrogenic mesenchyme. As an embryonic inducer, it triggers the development of all of the nephron anlagen [2]. In the ampullar neck, frequent cell divisions are observed which lead to a tubular elongation. The shaft shows a clear-cut transition zone. According to morphological and immunohistochemical criteria, here the first P and IC cells become visible [3, 4]. Their function is to regulate the Na^+/K^+ balance [5], the water transport [6], and the acid-base status of the body fluid [7]. The mechanisms by which different CD cell types develop are still unknown. Possible mechanisms are signals from the extracellular matrix [8, 9] and the influence of soluble growth factors or hormones [10–12].

We use the ampullar CD epithelium of the neonatal rabbit kidney as a model to investigate the mechanisms

KARGER
E-Mail karger@karger.ch
Fax + 41 61 306 12 34
http://www.karger.ch

1018–7782/97/0055–0414$15.00/0

This article is also accessible online at:
http://BioMedNet.com/karger

Prof. Will W. Minuth
Department of Anatomy, University of Regensburg
Universitätsstrasse 31
D–93053 Regensburg (Germany)
Fax 941 943 2868

involved in cellular differentiation [13]. From the medulla up to the ampulla the CD consists of a polarized epithelium. With respect to protein expression, it contains portions with embryonic, maturing, and differentiated CD cells [14]. Not only in the organ, but also under in vitro conditions the CD development can be investigated, since isolation and culture of the embryonic epithelia are easy to perform [15]. Previous experiments showed that CD development can be experimentally modulated [16]. Presuppositions are an improved culture technique [1, 14], a suitable extracellular matrix [4, 8, 17], and soluble differentiation factors [1, 10, 11]. In the present paper, we show that additionally the extracellular electrolyte composition has an important influence on CD differentiation.

Materials and Methods

Tissue Isolation and Generation of an Embryonic CD Epithelium

Cortical explants from the kidneys of newborn New Zealand rabbits were isolated according to methods described earlier [15]. The explants were mounted in sterile tissue carriers (fig. 1a; Minucells and Minutissue, Bad Abbach, Germany) which were placed in 24-well culture dishes (Greiner, Frickenhausen, Germany). The explants consisted of a piece of capsula fibrosa with adherent CD ampullae, S-shaped bodies, and nephrogenic blastema. During the culture of these explants in Iscove's modified Dulbecco's medium (IMDM) [18] (Gibco BRL Life Technologies, Eggenstein, Germany) and 10% fetal bovine serum (Boehringer Mannheim, Germany), an outgrowth of cells from the CD ampullae was observed. Within 24 h after the initiation of culture, the entire surface of the explant, 6 mm in diameter, was covered by polarized CD epithelium. Culture for the 1st day was carried out in a tissue incubator (Heraeus, Hanau, Germany) at 37°C in a humidified atmosphere containing 5% C_2O/95% air.

Perfusion Culture of Embryonic CD Epithelia in a Gradient Container

24 h after initiation of the culture, the epithelia were transferred to gradient containers (fig. 1b, c; Minucells and Minutissue) [19] to create improved culture conditions. Fresh medium was superfused at the luminal and basal sides of the epithelia for the culture period of

Fig. 1. Gradient perfusion culture with a renal CD epithelium. **(a)** The renal explant (E), 6 mm in diameter, is mounted in a tissue carrier. **b** The carrier is transferred to a gradient perfusion container which is connected to a luminal and a basal perfusion line. A peristaltic pump with two channels transports the media. **c** The closed gradient container allows luminal and basal perfusion with two different media. **b, c** Original size.

Table 1. Analytical parameters of neonatal rabbit serum, standard IMDM, IMDM plus NaCl, and IMDM plus NaCl/Na-gluconate-adapted medium for culturing embryonic CD epithelia

	Serum	IMDM	IMDM + 12 mmol/l NaCl	IMDM + 12 mmol/l NaCl/ 17 mmol/l Nagluconate
Ca^{2+}	1.66±0.46	1.15±0.27	1.15±0.1	1.15±0.03
K^+	6.04±1.7	4.25±0.1	4.25±0.04	4.25±0.04
Cl^-	99±5.8	85.1±1.0	98.7±1.4	98.5±1.2
Na^+	137±5.7	112.3±1.6	126.4±1.7	136.9±1.0
n	17	40	31	32

Values expressed as millimoles per liter, mean values ± half of range are shown.

13 days. The volume in the luminal and basal compartment of the gradient container was 1 ml each. The container was placed on a 37°C heating plate (Medax, Kiel, Germany). The culture medium was continuously perfused at a rate of 1 ml/h with an IPC N8 peristaltic pump (Ismatec, Wertheim, Germany). Antibiotic-antimycotic solution (1 ml/100 ml) and aldosterone (1×10^{-7} mmol/l), both from Sigma-Aldrich-Chemie (Deisenhofen, Germany), were added to all culture media. Since the culture system runs outside an incubator, all of the media contained 75 mmol/l HEPES to maintain a constant pH under laboratory air atmosphere.

Parameters of the Culture Medium

Commercially available IMDM [18] (Gibco BRL Life Technologies) was the standard medium. Culture parameters (pH, pCO_2, pO_2, lactate, osmolarity) and electrolyte concentrations (Na^+, K^+, Cl^-, soluble and unbound Ca^{2+}) of IMDM or neonatal rabbit serum specimens were determined within an undiluted 200 µl sample by a Stat Profile 9 Plus analyzer according to the manufacturer's instructions (Nova Biomedical, Rödermark, Germany; table 1). Solutions with defined electrolyte concentrations served as controls. Samples of the culture medium were taken from the inflow and outflow, and from the luminal and basal sides of the gradient container. The perfusion rate was controlled daily. Only sealing epithelia were harvested which were grown under a continuous medium flow at the luminal and basal sides.

Experimental Design

First series (fig. 3a): IMDM (table 1) was superfused at the luminal and basal side of the epithelia for the whole culture period of 13 days (fig. 3a).

Second series (fig. 4a): IMDM (table 1) was superfused at the luminal side of the epithelia for the whole culture period. At the basal side of the epithelia IMDM was altered by the addition of 12 mmol/l NaCl to give the parameters shown in table 1.

Third series (fig. 5a): IMDM (table 1) was superfused at the luminal side of the epithelia for the whole culture period. At the basal side of the epithelia IMDM was altered by the addition of 12 mmol/l NaCl and 17 mmol/l Na-gluconate to give the parameters shown in table 1.

Fourth series – comparison of different electrolytes (fig. 6): IMDM (table 1) was superfused at the luminal side of the epithelia for the whole culture period. At the basal side standard IMDM without additional electrolytes was used (fig. 6a). IMDM was altered by the addition of 12 mmol/l LiCl (fig. 6b), 12 mmol/l NaCl (fig. 6c), or 12 mmol/l KCl (fig. 6d).

NaCl, LiCl, KCl, and Na-gluconate were obtained from Sigma-Aldrich-Chemie.

Immunohistochemistry

The cultured tissues were taken out of the carriers, embedded in Tissue Tec (Miles Inc., Elkhart, Ind., USA), and frozen in liquid nitrogen. Then the tissue block had to be orientated so that exact cross sections of the epithelium could be performed. For microscopical examination of cultured epithelia and for immunohistochemical detection of CD proteins, 7-µm cryosections were prepared with a Cryostat HM 500 (Microm, Walldorf, Germany). Sections were stained with toluidine blue solution for control (fig. 2a). The following antibodies were used for labeling: monoclonal antibody (mab) 703 recognizes P cells, while mab 503 detects IC cells, as shown in earlier investigations [20]. Mab $P_{CD}9$ localizes a 32/39-kD protein on all of the neonatal CD cells [21]. Immunolabeling was started by fixing the cryosections for 10 min in ice-cold ethanol as described earlier [22]. 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 in order to saturate unspecific binding sites. The primary antibodies mab 703 and mab 503 (each of them diluted 1:100 in blocking buffer) and mab $P_{CD}9$ (undiluted hybridoma supernatant) were incubated for 1.5 h. After several rinses with PBS/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., USA) diluted 1:200 in blocking buffer. After being rinsed several times in PBS/1% BSA the specimens were coincubated for 45 min with a peanut agglutinin (PNA)-rhodamine conjugate (Vector, Burlingame, Vt., USA) diluted 1:2,000 in PBS to detect β-type IC cells [3]. In order to ensure the specifity of the antibody labeling, different controls were included in the experiments. Preimmune serum as well as irrelevant primary antibodies were applied. Furthermore, control sections were incubated with the detecting secondary antibody. None of the control sections showed any positive reaction on the cultured CD epithelia.

To label the nuclei in the epithelium 4 µg/ml propidium iodide (Sigma-Aldrich-Chemie, Deisenhofen, Germany) solution in PBS was used. Following several washes with PBS, the sections were embedded in FITC guard (Testoc, Chicago, Ill., USA) and examined using an Axiovert 35 microscope (Zeiss, Oberkochen, Germany).

Evaluation

To determine the degree of immunopositive cells in the cultured epithelia, a double-labeling procedure was applied. The epithelia were first labeled with the nuclear marker propidium iodide (fig. 2b) and then with a cellular marker such as mab 503, 703, or $P_{CD}9$ (fig. 2d). By this method the amount of mab-labeled and mab-unlabeled cells within the epithelium could easily be determined. The mean of immunopositive cells is given in the individual experimental series. In total more than 100 epithelia were examined from gradient perfusion culture experiments for the present investigation. Each treatment was repeated three times, and at least five epithelia were analyzed per experimental series.

Results

Culture Strategy

Embryonic CD epithelium was surgically isolated from the cortex of neonatal rabbit kidney by stripping off the capsula fibrosa [15]. The thin explants were mounted in tissue carriers so that the embryonic CD epithelium could develop within its original extracellular matrix environment (fig. 1a, 2a). To mimic a natural fluid exchange, the tissue carriers with the epithelia were placed in gradient containers (fig. 1b, c). By this method culture media could be superfused at both sides of the epithelia. It was possible to superfuse either the same medium (fig. 3a), or it was possible to keep the tissue in a gradient with different media (fig. 4a, 5a).

Microscopic Analysis of Cultured CD Epithelia

We examined the histological appearance of the cultured epithelia after they had been exposed to a constant medium flow in the gradient container for a period of 13 days (fig. 2) [19]. It was found that over a diameter of 6 mm the surface of the explant was completely covered by an epithelium (fig. 2a). This result was obtained in the first experimental series when standard IMDM was superfused on the apical and basal sides of the epithelium (fig. 3). The same good morphology was also observed when IMDM at the basal side contained additional 12 mmol/l NaCl (fig. 4) or 12 mmol/l NaCl in combination with 17 mmol/l Na-gluconate (fig. 5). Numerous experiments revealed that it is possible to culture an embryonic CD epithelium in an electrolyte gradient over a relatively long period of time. Labeling the epithelia with the proliferation marker Ki 67 (Dianova, Hamburg, Germany) was negative (fig. 2c), showing that the cultured tissue had reached a postmitotic state which is typical for matured kidney epithelia.

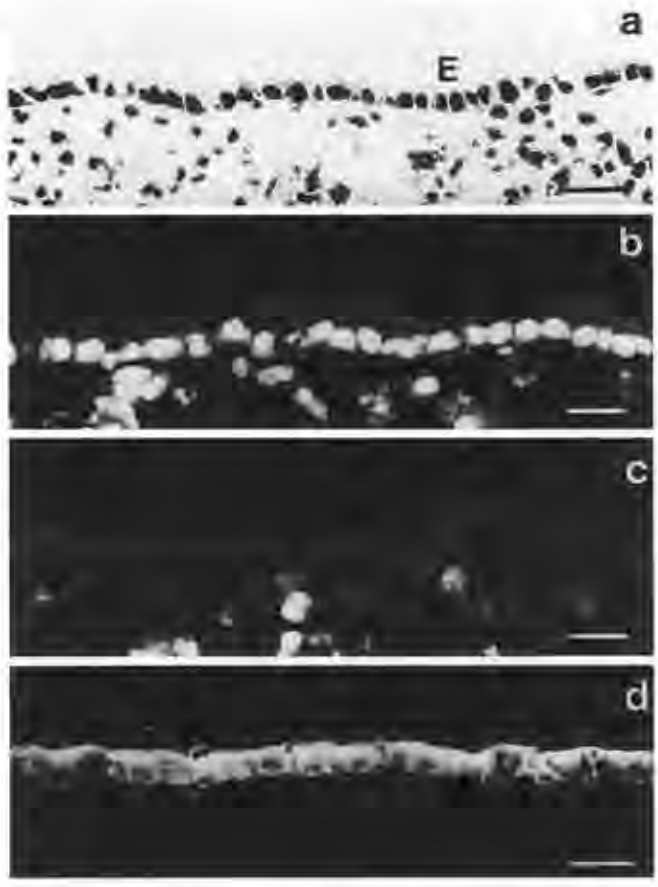
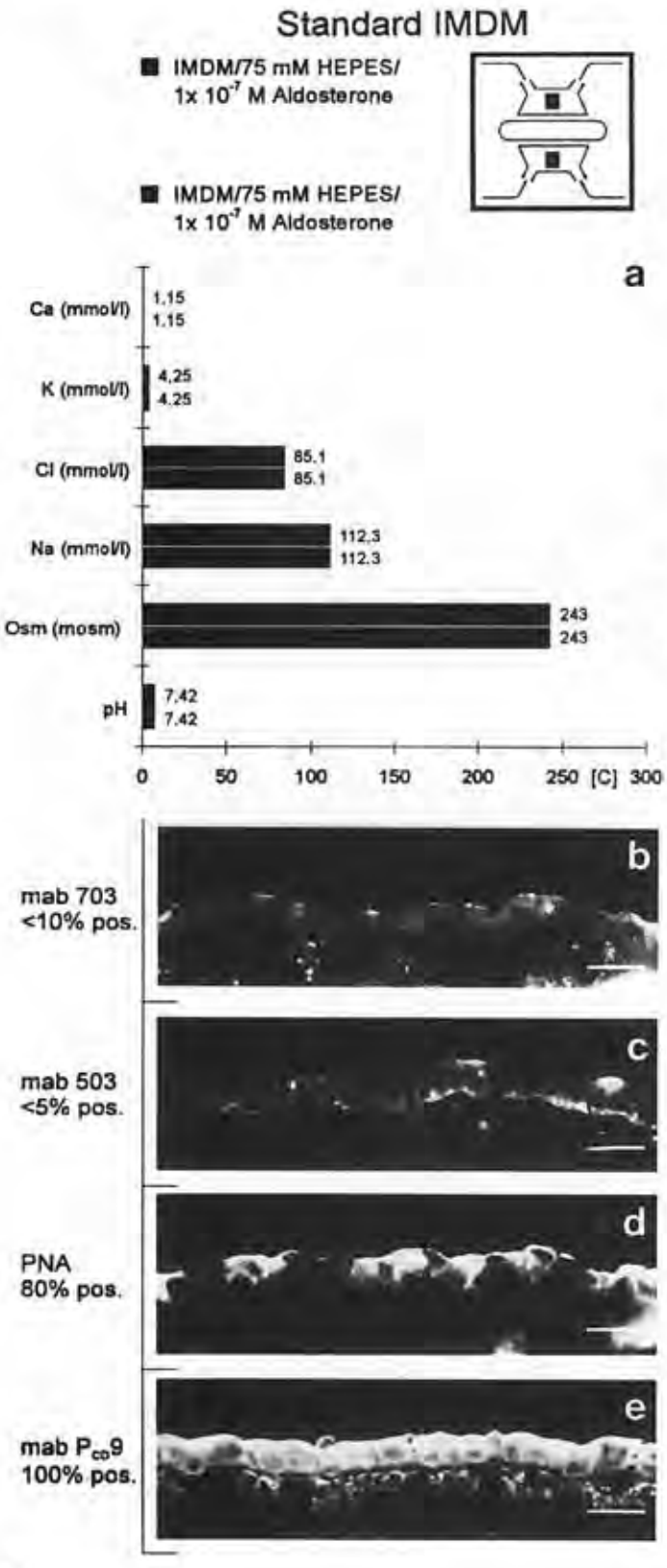


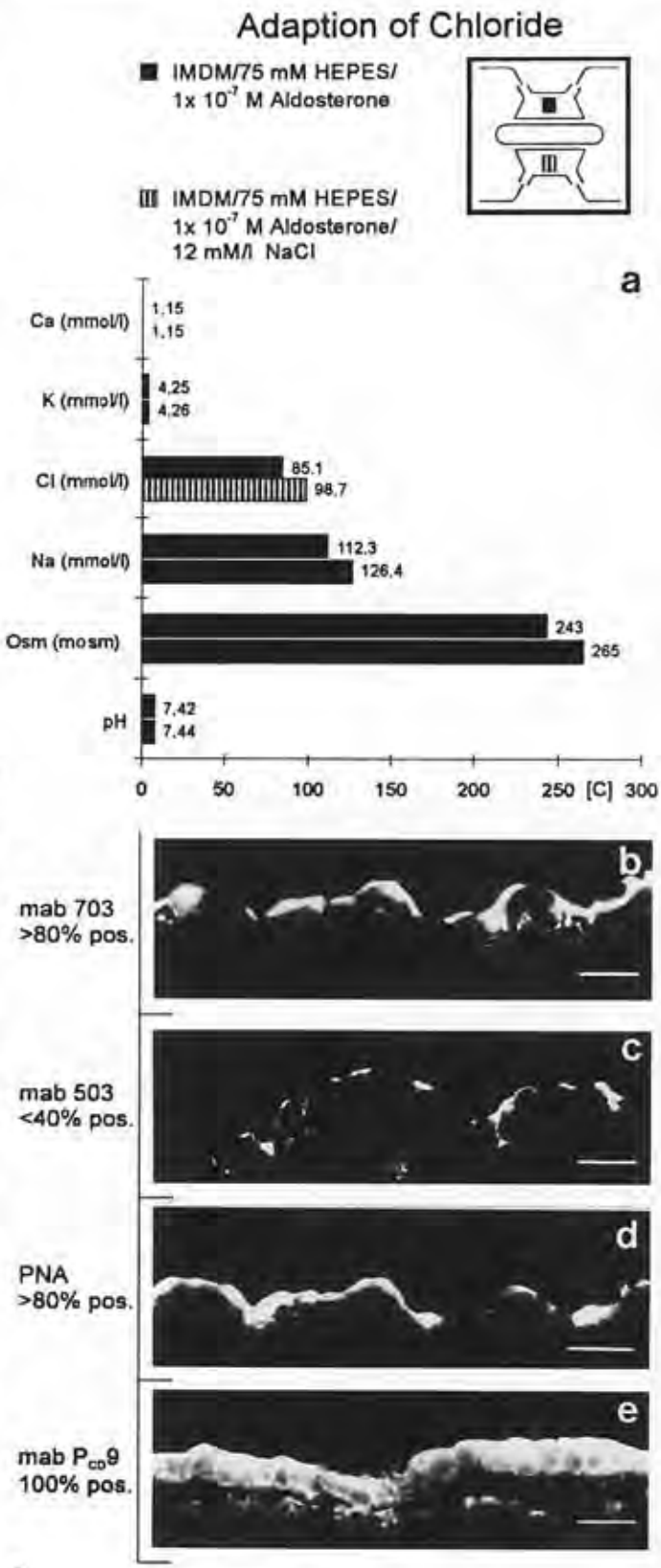
Fig. 2. Microscopy and fluorescence microscopy of renal CD epithelium (E) kept for 13 days in a gradient perfusion container. **a** The cryosection of a cultured CD epithelium demonstrates that a closed cell monolayer has been established which tolerates the medium flow in the gradient container. **b** Incubation with propidium iodide shows the number of nuclei and thus the number of cells within the epithelia. Not only nuclei from the epithelium, but also nuclei from interstitial cells beyond the basement membrane are labeled by the marker. **c** Immunohistochemistry with the proliferation marker mab Ki 67 is negative, indicating an absence of mitotic activity in the cultured epithelium, but not in the interstitial cells. **d** Immunolabeling with mab $P_{CD}9$ shows 100% positive cells within the epithelium. The ratio between labeled nuclei (**b**) and immunopositive cells (**d**) within the epithelium indicates the percentage of reacting cells. Control incubations for immunohistochemistry revealed the same unlabeled pattern as seen in **c**. The bars represent 20 μm.

Electrolytes Modulate Protein Expression in Embryonic CD Epithelia

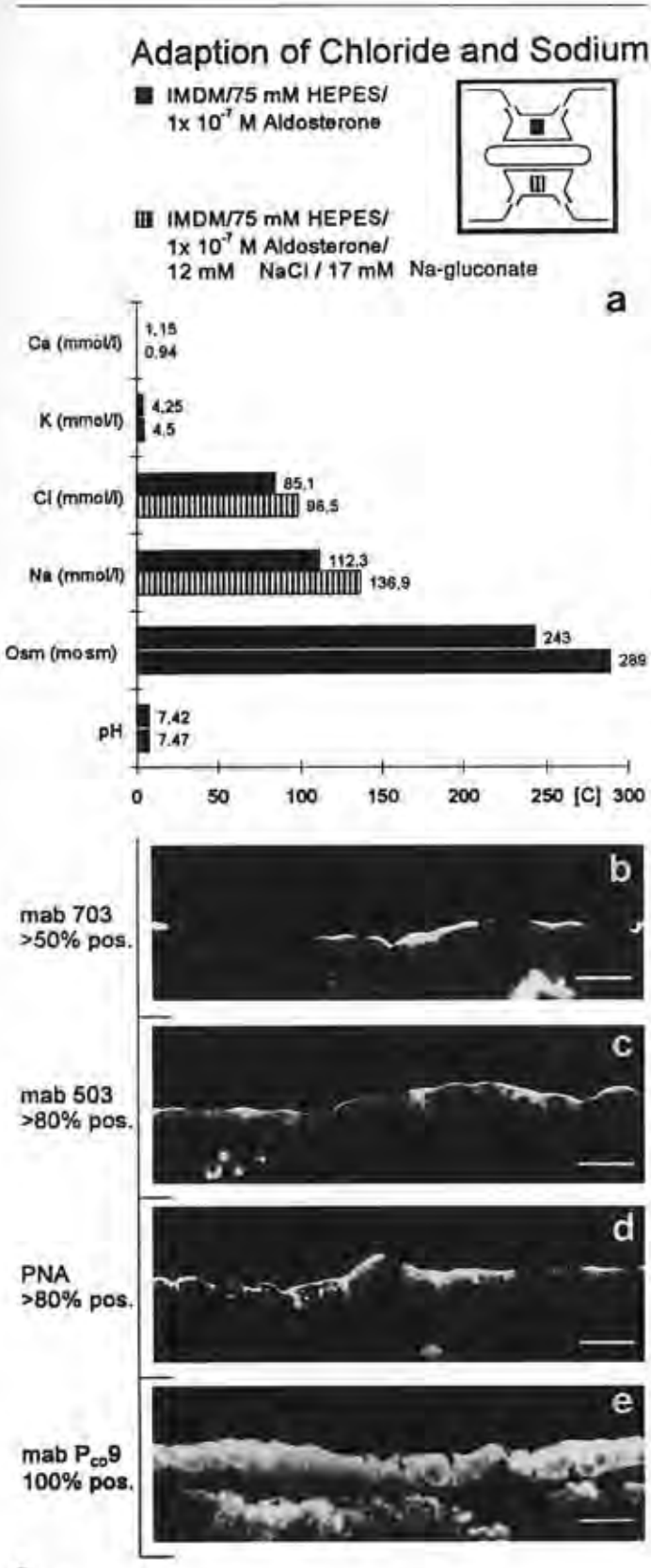
We analyzed whether the composition of the extracellular fluid environment contributes to the transition from an embryonic to a matured CD cell. The primary appearance of CD features was tested by immunohistochemical methods at the end of culture (fig. 3–5).


Standard IMDM
IMDM/75 mM HEPES/
1x 10⁻⁷ M Aldosterone
IMDM/75 mM HEPES/
1x 10⁻⁷ M Aldosterone
a
Ca (mmol/l)
1,15
1,15
K (mmol/l)
4,25
4,25
Cl (mmol/l)
85,1
85,1
Na (mmol/l)
112,3
112,3
Osm (mosm)
243
243
pH
7,42
7,42
0
50
100
150
200
250 [C] 300
mab 703
<10% pos.
b
mab 503
<5% pos.
c
PNA
80% pos.
d
mab P$_{cd}$9
100% pos.
e

3


Adaption of Chloride
IMDM/75 mM HEPES/
1x 10⁻⁷ M Aldosterone
IMDM/75 mM HEPES/
1x 10⁻⁷ M Aldosterone/
12 mM/l NaCl
a
Ca (mmol/l)
1,15
1,15
K (mmol/l)
4,25
4,26
Cl (mmol/l)
85,1
98,7
Na (mmol/l)
112,3
126,4
Osm (mosm)
243
265
pH
7,42
7,44
0
50
100
150
200
250 [C] 300
mab 703
>80% pos.
b
mab 503
<40% pos.
c
PNA
>80% pos.
d
mab P$_{cd}$9
100% pos.
e

4



In the first set of experiments the embryonic CD epithelia were cultured in a gradient container and superfused on the luminal and basal sides with standard IMDM for 13 days (fig. 3a, table 1). The epithelia showed less than 10% mab 703 (fig. 3b) and less than 5% mab 503 (fig. 3c) positive cells. Since aldosterone [19] was present in all culture media, PNA labeling (fig. 3d) consistently revealed more than 80% positive cells within the cultured epithelium. Immunolabeling with mab $P_{CD}9$ (fig. 3e) always showed 100% positive cells.

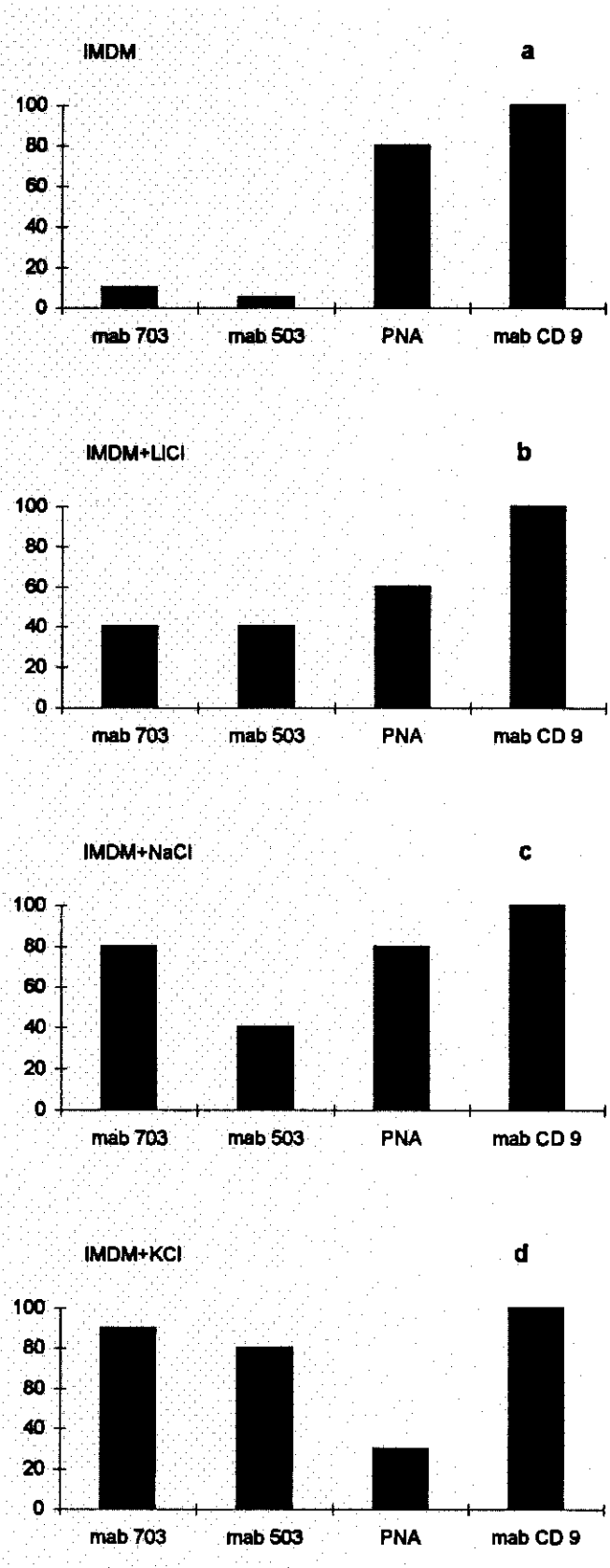
In the second set of experiments the embryonic epithelia were superfused at the apical side with standard IMDM and at the basal side with IMDM containing addional 12 mmol/l NaCl (fig. 4a, table 1). In this set of experiments more than 80% of the cells showed an intensive mab 703 (fig. 4b) and less than 40% of the cells a faint mab 503 immunolabeling (fig. 4c). Again, PNA labeling was detected on more than 80% of the cells (fig. 4d). Mab $P_{CD}9$ labeling was found on all of the CD cells (fig. 4e). Compared to the first series (fig. 3), the second series showed that addition of 12 mmol/l NaCl to the superfusion medium at the basal side of the epithelia led to increases of mab 703 (fig. 4b) and mab 503 (fig. 4c) positive cells.

In the third experimental series standard IMDM was superfused at the apical side of the epithelia, while at the basal side IMDM with additional 12 mmol/l NaCl and 17 mmol/l Na-gluconate was administered (fig. 5a, table 1). This electrolyte alteration resulted in the same Na^+ and Cl^- concentrations as observed in the serum of neonatal

Fig. 3. Culture of embryonic CD epithelium in a gradient container for 13 days. Standard IMDM was used on both the luminal and basal sides (**a**). Immunofluorescence microscopy revealed less than 10% mab 703 (**b**) and less than 5% mab 503 (**c**) positive cells. In contrast, PNA was always on 80% (**d**) and mab $P_{CD}9$ labeled consistently 100% of the cells (**e**). The bars in **b–e** represent 20 μm.

Fig. 4. Culture of embryonic CD epithelia in a gradient container for 13 days. On the luminal side standard IMDM and on the basal side standard IMDM containing additional 12 mmol/l NaCl (**a**). Immunofluorescence microscopy revealed more than 80% mab 703 (**b**) and more than 40% mab 503 (**c**) positive cells. In contrast, PNA was always on more than 80% (**d**), and mab $P_{CD}9$ consistently labeled 100% of the cells. The bars in **b–e** represent 20 μm.

Fig. 5. Culture of embryonic CD epithelium in a gradient container for 13 days. On the luminal side standard IMDM was used and on the basal side IMDM with additional 12 mmol/l NaCl and 17 mmol/l Na-gluconate (**a**). Immunofluorescence microscopy revealed more than 50% mab 703 (**b**) and more than 80% mab 503 (**c**) positive cells. In contrast, PNA was always on more than 80% (**d**) and mab $P_{CD}9$ labeled consistently 100% of the cells (**e**). The bars in **b–e** represent 20 μm.



rabbits. More than 50% of the cells showed an intensive immunolabeling with mab 703 (fig. 5b) and more than 80% with mab 503 (fig. 5c). Again, PNA binding (fig. 5d) and mab $P_{CD}9$ (fig. 5e) immunolabeling was unchanged as compared to the first (fig. 3) and second (fig. 4) experimental setup. Thus, increasing concentrations of Na^+ did not further increase the amount of mab 703, but the amount of mab 503 positive cells.

The fourth set of experiments made clear whether the primary development of CD cell features in the embryonic epithelia is only caused by increased concentrations of NaCl (fig. 4, 6c) or whether also 12 mmol/l LiCl (fig. 6b) or KCl (fig. 6d) had the same effect. Superfusion of standard IMDM for control at the basal and apical sides of the epithelia caused less than 10% mab 703, less than 5% mab 503, and 100% mab $P_{CD}9$ positive cells (fig. 6a). 80% of the cells showed PNA binding. Then at the basal side IMDM was altered with additional 12 mmol/l LiCl (fig. 6b). As compared to controls (fig. 6a) the epithelia showed more mab 703 and mab 503 positive cells. 40% mab 703, 40% mab 503, and 100% mab $P_{CD}9$ labeled cells were observed. On 60% of the cells PNA binding was found. In series with IMDM containing additional 12 mmol/l NaCl, 80% mab 703, 40% mab 503, and 100% mab $P_{CD}9$ positive cells could be observed (fig. 6c). 80% of the cells revealed a PNA binding. Replacement of NaCl with 12 mmol/l KCl led to 90% mab 703 and 80% mab 503 positive cells (fig. 6d). The amount of PNA-binding cells was found to be decreased to 30%. The number of mab $P_{CD}9$-binding cells was not influenced. Thus, the experiments showed that IMDM altered by additional 12 mmol/l NaCl, LiCl or KCl had an influence on the development of cell features in embryonic CD epithelia.

Discussion

From Proliferation to Terminal Differentiation

Functional development of the embryonic CD starts in the ampullar neck. The cells at this site proliferate, and the tubule reaches its final length. Beyond this zone cell

Fig. 6. Electrolytes influence differentiation in embryonic CD epithelium cultured in a gradient container for 13 days. **a** Control: on the luminal and basal sides standard IMDM was used. **b–d** Experimental series: on the luminal side standard IMDM and on the basal side standard IMDM with additional 12 mmol/l LiCl (**b**), NaCl (**c**), or KCl (**d**) was administered. Compared to controls (**a**) the most mab 703 and mab 503 positive cells were found after NaCl (**c**) and KCl (**d**) and not after LiCl (**b**) treatment.

divisions become rare and terminal differentiation appears [1]. An epithelial barrier is formed. Tight junctions are sealed, pumps and channels are synthesized. While each of the nephron portions consists of a homogeneous cell population, the renal CD develops into a heterogeneously composed epithelium consisting of P and different types of IC cells [3, 4, 7]. We were interested in the mechanism by which the different cell types arise. Because of the complexity of the kidney, we use an in vitro model [15]. However, previous experiences with cultured renal epithelia have shown that investigations are hindered by cellular dedifferentiation [23, 24]. This fact made it necessary to follow an innovative experimental culture protocol [25]. We kept the embryonic epithelia on tissue carriers and within gradient culture containers. Fresh medium was permanently superfused on the apical and basal sides of the embryonic epithelia (fig. 1).

Control of Differentiation at Different Cellular Levels

In general, differentiation of cultured epithelia can be influenced not only by the extracellular matrix [8, 9, 26] and by humoral factors [10, 12, 27], but also by a continuous supply of nutrients or even by the permanent elimination of harmful metabolic products [28]. Previous experiments have further shown that changes in the extracellular environment modulate metabolic processes [29–31] and stimulate proliferation [32–34] in adult cells. Thus, the electrolyte environment may also have an influence on the differentiation of embryonic cells. For that reason we exposed embryonic CD epithelia to different electrolyte environments and registered the primary appearance of typical features (fig. 3–6). In the present experiments we were able to show for the first time that the electrolyte environment plays an important role in embryonic CD differentiation.

Electrolyte Modulation Influences Facultative Protein Expression

In previous [1, 14, 19] and in the present experiments IMDM was used as the standard culture medium. It contains 112 mmol/l Na^+ and 85 mmol/l Cl^- (table 1) and delivers excellent conditions for proliferating cells in culture [18]. In conventional cultures epithelia are exposed at the luminal and basal sides to the same medium. However, our experimental interest was to expose the embryonic CD epithelia to a luminal-basal gradient during culture. For that reason the embryonic CD epithelia were exposed to standard IMDM at the luminal side and to an electrolyte environment similar to the serum of neonatal rabbits at the basal side (fig. 4–6, table 1). We found that modifying the luminal-basal electrolyte environment influences the primary protein expression in the embryonic CD epithelia. As compared to controls (fig. 3), it was demonstrated that it is not an overall upregulation of protein expression. The expression of proteins recognized by the monoclonal antibodies 703 or 503 is triggered by the extracellular electrolyte environment (fig. 4–6). The development of PNA binding is not influenced by IMDM containing additional 12 mmol/l NaCl (fig. 4d, 5d), but downregulated by IMDM with additional 12 mmol/l LiCl (fig. 6b) or KCl (fig. 6d). In contrast, the expression of the CD protein $P_{CD}9$ occurs independently and is not influenced by the electrolyte environment (fig. 3e, 4e, 5e).

How Essential Might an Electrolyte Sensor Be for Differentiation?

The presented results raise the question of which type of an electrolyte sensor for cell differentiation exists in the embryonic CD cells and influences the expression of individual cell features. Such a sensor might act on the basis of a specific influence such as the Na^+ or Cl^- concentration or a more unspecific signal such as the ratio of the intra-/extracellular electrolytes. Culture experiments of the embryonic CD epithelium in IMDM containing additional 12 mmol/l LiCl, NaCl, or KCl (fig. 6) led to the conclusion that the development is not influenced by a specific Na^+ effect [36]. A hypothetical sensor may have a direct influence on gene expression [37], or it may act through an indirect pathway which first stimulates a second-messenger system. In contrast to earlier investigations [32, 33], we found that the electrolyte environment does not stimulate the mitotic activity of the embryonic CD cells (fig. 2c) [35]. It remains to investigate whether there is a coherence to previous described experiments with cultured MDCK cells under pH variations and changes in osmolarity [31, 38]. Originally we expected that increasing concentrations of NaCl in the culture medium would raise more P than IC cells, since abundant Na^+ increases the number of Na channels during kidney development [39]. However, we now know that both P and IC cell features in the embryonic CD epithelia are influenced by varying concentrations of electrolytes.

Acknowledgments

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

References

1 Aigner J, Kloth S, Minuth WW: Transitional differentiation patterns of principal and intercalated cells during renal collecting duct development. Epithelial Cell Biol 1995;4:121–130.
2 Saxén L: Organogenesis of the kidney; in Barlow PW, Green PB, Wylie CC (eds): Developmental and Cell Biology Series. Cambridge, Cambridge University Press, 1987, vol 19.
3 Satlin LM, Matsumoto T, Schwartz GJ: Postnatal maturation of rabbit renal collecting duct. III. Peanut lectin binding intercalated cells. Am J Physiol 1992;262:F199–F208.
4 Kim J, Tisher CC, Madsen KM: Differentiation of intercalated cells in developing rat kidney: An immunohistochemical study. Am J Physiol 1994;266:F977–F990.
5 Rossier BC, Paccolat MP, Verrey F, Kraehenbühl JP, Geering K: Mechanism of action of aldosterone: A pleiotropic response. Horm Cell Regul 1985;9:209–225.
6 Naruse M, Yoshitomi K, Hanaoka K, Imai M, Kurokawa K: Electrophysiological study of luminal and basolateral vasopressin in rabbit cortical collecting duct. Am J Physiol 1995;268: F20–F29.
7 Emmons C, Kurtz I: Functional characterization of three intercalated cell subtypes in the rabbit outer cortical collecting duct. J Clin Invest 1994;93:417–423.
8 Ekblom P: Formation of basement membranes in the embryonic kidney: An immunohistological study. J Cell Biol 1981;91:1–10.
9 Sorokin L, Ekblom P: Development of tubular and glomerular cells of the kidney. Kidney Int 1992;41:657–664.
10 Chailler P, Ferrari J, Brière N: Fetal mouse kidney maturation in vitro: Coordinated influences of epidermal growth factor, transferrin and hydrocortisone. Anat Embryol (Berl) 1991; 184:319–329.
11 Segal R, Fine LG: Polypeptide growth factors and the kidney. Kidney Int 1989;36:2–10.
12 Weller A, Sorokin L, Illgen EM, Ekblom P: Development and growth of mouse embryonic kidney in organ culture and modulation of development by soluble growth factor. Dev Biol 1991;144:248–261.
13 Vehaskari VM: Ontogeny of cortical collecting duct sodium transport. Am J Physiol 1994; 267:F49–F54.
14 Aigner J, Kloth S, Kubitza M, Kashgarian M, Dermietzel R, Minuth WW: Maturation of renal collecting duct cells in vivo and under perifusion culture. Epithelial Cell Biol 1994;3:70–78.
15 Minuth WW: Neonatal rabbit kidney cortex in culture as tool for the study of collecting duct formation and nephron differentiation. Differentiation 1987;36:12–22.
16 Minuth WW, Gilbert P, Gross P: Appearance of specific proteins in the apical plasma membrane of cultured renal collecting duct principal cell epithelium after chronic administration of aldosterone and arginine vasopressin. Differentiation 1988;38:194–202.
17 Evan AP, Satlin LM, Gattone VH, Connors B, Schwartz GJ: Postnatal maturation of rabbit renal collecting duct. II. Morphological observations. Am J Physiol 1991;261:F91–F107.
18 Iscove NN, Melchers F: Complete replacement of serum by albumin, transferrin and soybean lipids in cultures of lipopolysaccharide-reactive B lymphocytes. J ExptMed 1978;147:923–933.
19 Minuth WW, Aigner J, Kloth S, Steiner P, Tauc M, Jennings ML: Culture of embryonic renal collecting duct epithelium in a gradient container. Pediatr Nephrol 1997;11:140–147.
20 Tauc M, Koechlin N, Jamous M, Ouaghi EM, 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 1992;76:55–65.
21 Kloth S, Gmeiner T, Aigner J, Jennings ML, Minuth WW: Transitional stages of renal collecting duct development. Differentiation, resubmitted.
22 Kloth S, Aigner J, Brandt E, Moll R, Minuth WW: Histochemical markers reveal an unexpected heterogenous composition of the renal embryonic collecting duct epithelium. Kidney Int 1993;44:527–536.
23 Koechlin N, Pisam M, Poujeol P, Tauc M, Rambourg A: Conversion of a rabbit proximal convoluted tubule (PCT) into a cell monolayer: Ultrastructural study of cell dedifferentiation and redifferentiation. Eur J Cell Biol 1991;54: 224–236.
24 Minuth WW, Aigner J, Kubat B, Kloth S: Improved differentiation of renal tubular epithelium in vitro: Potential for tissue engineering. Exp Nephrol 1997;5:10–17.
25 Minuth WW, Dermietzel R, Kloth S, Hennerkes B: A new method culturing renal cells under permanent superfusion and producing a luminal-basal medium gradient. Kidney Int 1992;41:215–219.
26 Zuk A, Matlin KS, Hay ED: Type I collagen gel induces Madin-Darby canine kidney cells to become fusiform in shape and lose apical-basal polarity. J Cell Biol 1989;108:903–919.
27 Handler JS, Preston AS, Steele RE: Factors affecting the differentiation of epithelial transport and responsiveness to hormones. Fed Proc 1984;43:2221–2224.
28 Jauregi HO, Naik S, Santangini H, Pan J, Trenkler D, Mullon C: Primary cultures of rat hepatocytes in hollow fiber chambers. In Vitro Cell Dev Biol 1993;30A:23–29.
29 Yamaji Y, Moe OW, Miller RT, Alpern RJ: Acid activation of immediate early genes in renal epithelial cells. J Clin Invest 1994;94: 1297–1303.
30 Logan JL: Studies on renal growth regulation by urea and ammonia in normal rat kidney cells. Nephron 1995;71:433–441.
31 Pfaller W, Steigner W, Gstraunsthaler G, Kersting U, Oberleithner H: Low pH and hyperosmolarity determine the differentiation of MDCK cells typical for the outer medullary collecting duct. Cell Physiol Biochem 1992;68: 231–243.
32 Walsh-Reitz MM, Aithal HN, Toback FG: Na regulated growth of kidney epithelial cells induced by lowering extracellular K concentrations. Am J Physiol 1984;247:C321–C326.
33 Toback FG: Induction of growth in kidney epithelial cells in culture by Na. Proc Natl Acad Sci USA 1980;77:6654–6656.
34 Isabelle ME, Githens S, Moses RL, Bartell CK: Culture of rat renal medullary tissue in media made hyperosmotic with NaCl and urea. J Exp Zool 1994;269:308–318.
35 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 1991;138:867–873.
36 Davies J, Garrod DR: Induction of early stages of kidney tubule differentiation by lithium ions. Dev Biol 1995;167:50–60.
37 Yoshimi T, Nakamura M, Asano A: Expression of several muscle-specific genes during differentiation of cultured quail pineal body cells under artificial (high NaCl) conditions. Differentiation 1995;59:307–319.
38 Hayashi M, Iyori M, Yamaji Y, Saruta G: Effects of in vivo and in vitro alkali treatment of an intracellular pH regulation of OMCD cells. Am J Physiol 1993;265:F729–F735.
39 Satlin LM, Palmer LG: Apical Na^+ conductance in maturing rabbit principal cell. Am J Physiol 1996;270:F391–F397.