Kidney International, Vol. 39 (1991), pp. 1168–1175

Characterization of a mouse cortical collecting duct cell line

BARBARA A. STOOS, ANIKÓ NÁRAY-FEJES-TÓTH,[1] OSCAR A. CARRETERO, SADAYOSHI ITO, and GÉZA FEJES-TÓTH[1]

Henry Ford Hospital, Hypertension Research Division, Detroit, Michigan, USA

Characterization of a mouse cortical collecting duct cell line. A cortical collecting duct (CCD) cell line has been developed from a mouse transgenic for the early region of simian virus 40, Tg(SV40E)Bri/7. CCDs were microdissected and placed on collagen gels. Monolayers were subsequently subcultured onto permeable collagen membranes and maintained in serum-supplemented medium. One line, designated M-1, retained many characteristics of the CCD, including a typical epithelial appearance and CCD-specific antigens. M-1 cells, when grown in monolayers on permeable supports, exhibited a high transepithelial resistance (885.7 ± 109.6 ohms/cm^2) and developed a lumen negative transepithelial potential difference (PD) of −45.7 ± 3.5 mV. The associated short-circuit current (SCC) averaged 71.8 ± 10.3 μA/cm^2, and was reduced by 95% by luminal application of amiloride. The cultured cells responded to arginine vasopressin (AVP) with a significant increase in SCC. M-1 cells generated significant transepithelial solute gradients. After 24 hours incubation, the composition of the luminal (L) and basolateral (B) media (in mM) was: [Na^+], L = 106.7 ± 0.9 and B = 127.4 ± 0.4; [K^+], L = 8.6 ± 0.6 and B = 2.1 ± 0.3; [Cl], L = 68.6 ± 5.8 and B = 101.8 ± 6.6; [HCO_3], L = 15.5 ± 1.5 and B = 8.6 ± 1.2; while pH was 7.16 ± 0.03 at the luminal and 6.94 ± 0.03 at the basolateral side. The formation of these concentration gradients indicates that the CCD cultures absorb Na^+ and Cl^- and secrete K^+. Lactate accumulated predominantly at the basolateral side (L = 7.1 ± 0.44 and B = 17.5 ± 0.52 mM); osmotic concentration was 272 ± 1.4 at the luminal and 290 ± 3.0 mOsm/kg at the basolateral side. These data demonstrate that the M-1 cell line retains many phenotypic properties of the CCD epithelium and thus should prove useful as a model in studying mechanisms of ion transport in this segment.

The renal collecting duct (CD) plays a critical role in the fine regulation of salt, water, and acid-base balance. The main transport processes occurring in this segment are electrogenic sodium reabsorption, potassium secretion, and bidirectional transport of bicarbonate. Establishment of a CCD cell line would facilitate the study of mechanisms involved in these transport processes and their regulation.

Transepithelial transport has been successfully studied in several cell culture systems [c.f. 1]. Cell lines from defined nephron segments are particularly advantageous for a number of reasons: a) they provide a continuous source of readily available cells; b) transepithelial electrical and transport properties can easily be studied in an Ussing-type chamber when cells are cultured on permeable supports; c) variability between samples is minimal; and d) long-term environmental control is possible. If nephron-specific transport functions are to be studied successfully in vitro, cell lines retaining representative characteristics of the in vivo segment are necessary. While cell lines with representative characteristics of in vivo epithelia have been developed from amphibians [2, 3], those from mammals have not been as successful. In most previous studies with mammalian renal epithelial cell lines either the nephron segment from which the line originates has been unknown or some of the segment-specific functions were altered [4–7]. In the case of the cortical collecting duct (CCD), Arend et al [8] developed a rabbit cell line (RCCT-28A) which retains antigenic properties as well as the ability to respond to hormones specific to the CCD. However, RCCT-28A cells did not develop a tight epithelium, which limits their use as a model for transport studies.

This study describes the development of a mouse cell line (M-1) from the CCD. M-1 cells retain many differentiated transport functions, hormone responsiveness and CCD antigens and thus could serve as a valuable tool for the study of CCD transport in vitro under well-defined conditions.

Methods

Isolation of collecting ducts

Mice transgenic for the early region of SV40, Tg(SV40E)Bri7, [9, 10] were supplied by Ralph A. Brinster. A mouse was anesthetized with 20 mg/kg xylazine and 70 mg/kg ketamine i.p. The aorta was cannulated through an abdominal incision and both kidneys were perfused in situ with sterile medium. The kidneys were excised and sliced with a razor blade along the corticomedullary axis. The strips of renal tissue were transferred to Petri dishes containing Hank's balanced salt solution. The light portions of the collecting ducts were identified using criteria in [11]. Three tubules, 150 to 750 μm in lengths, were dissected under a stereomicroscope (SZH, Olympus, Overland, Kansas, USA) from the outer half of the cortex using sharpened forceps (Dumont No. 5; Fine Science Tools, Inc., Belmont, California, USA).

Culture of the CCD cells

Each microdissected tubule was placed in a 0.32 cm^2 well on top of a collagen gel (Vitrogen 100, Collagen Co, Palo Alto, California, USA). The tubules were incubated in RPMI 1640

[1] Present address: Department of Physiology, Dartmouth Medical School, Hanover NH, USA.

Received for publication July 20, 1990
and in revised form January 14, 1991
Accepted for publication January 15, 1991

containing 5% fetal bovine serum, 0.125% SerXtend (a serum extender from Hana Biologics, Alameda, California, USA), 1 mM pyruvate, 2 g/liter dextrose, 2 mM glutamine, 10^{-5} M 2-mercaptoethanol, 50 U/ml penicillin, 50 μg/ml streptomycin, 12 μg/ml tylosin, and 2% Ultroser G (a serum supplement from IBF Biotechnics, Garenne, France); this medium is subsequently referred to as medium A. The cultures were equilibrated with 5% CO_2-95% air and kept at 37°C. At confluence, the cells were subcultured onto either permeable collagen membranes (Cellagen units, ICN Biochemicals, Cleveland, Ohio, USA) with an active surface area of 0.6 cm^2 or cross-linked collagen microcarriers (Ventrex, Portland, Maine, USA). Subcultured cells were maintained either on medium A or on PC1 medium (Ventrex, Portland, Maine, USA) supplemented with 5% fetal bovine serum, 2 mM glutamine and antibiotics (subsequently referred to as medium B). The permeable collagen supports were placed in the central well of an organ culture dish and incubated with 400 and 800 μl medium at the luminal and basolateral side, respectively. To reduce evaporation and minimize contamination, sterile saline containing antibiotics was placed in the outer trough of the culture dish.

Cultures were subcultured by washing and then incubating the cells in Hank's balanced salt solution containing 20 mM Hepes and 0.1% collagenase (CLS II-Worthington, Freehold, New Jersey, USA). Cultures were kept on a shaker at 37°C until the collagen membranes or carriers were digested. Cells were dispersed by pipetting, washed twice and seeded onto collagen membranes with approximately 100,000 cells and microcarriers with 1.2×10^5 cells/mg.

Immunocytochemistry

Three monoclonal antibodies (MCAB), F_{13}/0121, ST.12 [12], and B63 [13], were used to characterize the cultured cells. Histochemistry was done on paraffin-embedded mouse kidney sections lightly fixed with periodate-lysine-paraformaldehyde. Slides were heated at 60°C for 20 minutes, deparaffinized in xylene, and rehydrated in a graded series of ethanol dilutions. The sections were covered with one of the three antibodies and incubated at room temperature for 90 minutes. Sections were washed and then incubated with fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse Ig (Boehringer Mannheim, Indianapolis, Indiana, USA) for 90 minutes at room temperature in the dark. After rinsing, slides were mounted in glycerol gelatin and inspected by epifluorescence microscopy.

M-1 cells cultured in slide wells were fixed in acetone and stained with the three antibodies as described above. In addition, cultured cells were stained for alkaline phosphatase activity, a proximal tubule marker [12], with an antibody against Tamm-Horsfall protein (Cappel, Cochranville, Pennsylvania, USA), a glycoprotein synthesized only by the thick ascending limb of the loop of Henle [14], with IVF12, a monoclonal antibody against human erythroid band 3 [15] and α-178, a polyclonal antibody against murine band 3 [16].

Microscopy

Monolayers grown on collagen membranes were observed under a Nikon phase-contrast microscope at magnifications of 300 to 600×. Cells cultured on Millipore filters were fixed in 3% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.0) for 24 hours at room temperature. The specimens were rinsed in the same buffer for five minutes at room temperature and placed in 0.1 M cacodylate buffer containing 1% osmium and 0.2 M sucrose for two hours. The samples were dehydrated in a series of ethanol washes, immersed in hexamethyldisilazane for five minutes, air-dried, mounted on aluminum stubs, coated with gold-palladium in a sputter coater and examined under a scanning electron microscope.

Analytical methods

Na^+ and K^+ concentrations were measured using ion-selective electrodes (NOVA Biomedical, Newton, Massachusetts, USA). Osmolality was determined by freezing-point depression. Samples of medium taken from the cultures were equilibrated with 5% CO_2 during measurements of H^+ concentration and prior to enzymatic determination of CO_2 concentration [17], from which HCO_3^- content was derived. Cl concentration was determined by its reaction with mercuric thiocyanate and lactate was measured enzymatically using reagents from Sigma Chemical Co. (St. Louis, Missouri, USA).

cAMP accumulation was determined using M-1 cells grown on microcarriers. Incubation with agonists (10^{-9} M AVP or 10^{-6} M bovine PTH) and assay of cAMP content by RIA was performed as described in detail elsewhere [18].

^{22}Na flux measurements

Lumen-to-bath flux of ^{22}Na was determined in M-1 monolayers grown on Cellagen membranes in medium B. Monolayers were incubated with ^{22}Na added to the luminal side only. After two hours media from both sides were collected and ^{22}Na associated radioactivity was measured in a gamma-scintillation counter.

Electrophysiology

Transepithelial potential difference (PD) and short-circuit current (SCC) were measured daily as described previously [19]. Electrical resistance (R) was calculated from the ratio of PD to SCC.

The effects of amiloride and AVP on transepithelial PD and SCC were determined in the following way. SCC was measured for two seconds at 30-second intervals. PD was measured during the 30-second intervals, thereby maintaining the culture in a predominantly open-circuit condition. Once baseline recordings had stabilized, we either added amiloride (20 μM) to the luminal side of the culture or AVP (10^{-9} M) to the basolateral side. Recordings were continued and subsequent values of SCC and PD were reported during the period of maximal deviation from baseline; the difference between subsequent and baseline values was considered to represent the response to the two compounds. In separate experiments, vehicle was added to the culture; however, there was no significant deviation from baseline within the time frame of the maximal responses.

Statistical evaluation

Values are reported as the mean ± standard error. Data were evaluated using Student's paired *t*-test.

Results

One of the three cell lines originating from the microdissected tubules (M-1) retained many of the characteristics of the original CCD through 32 passages; the other two cell lines (M-2 and

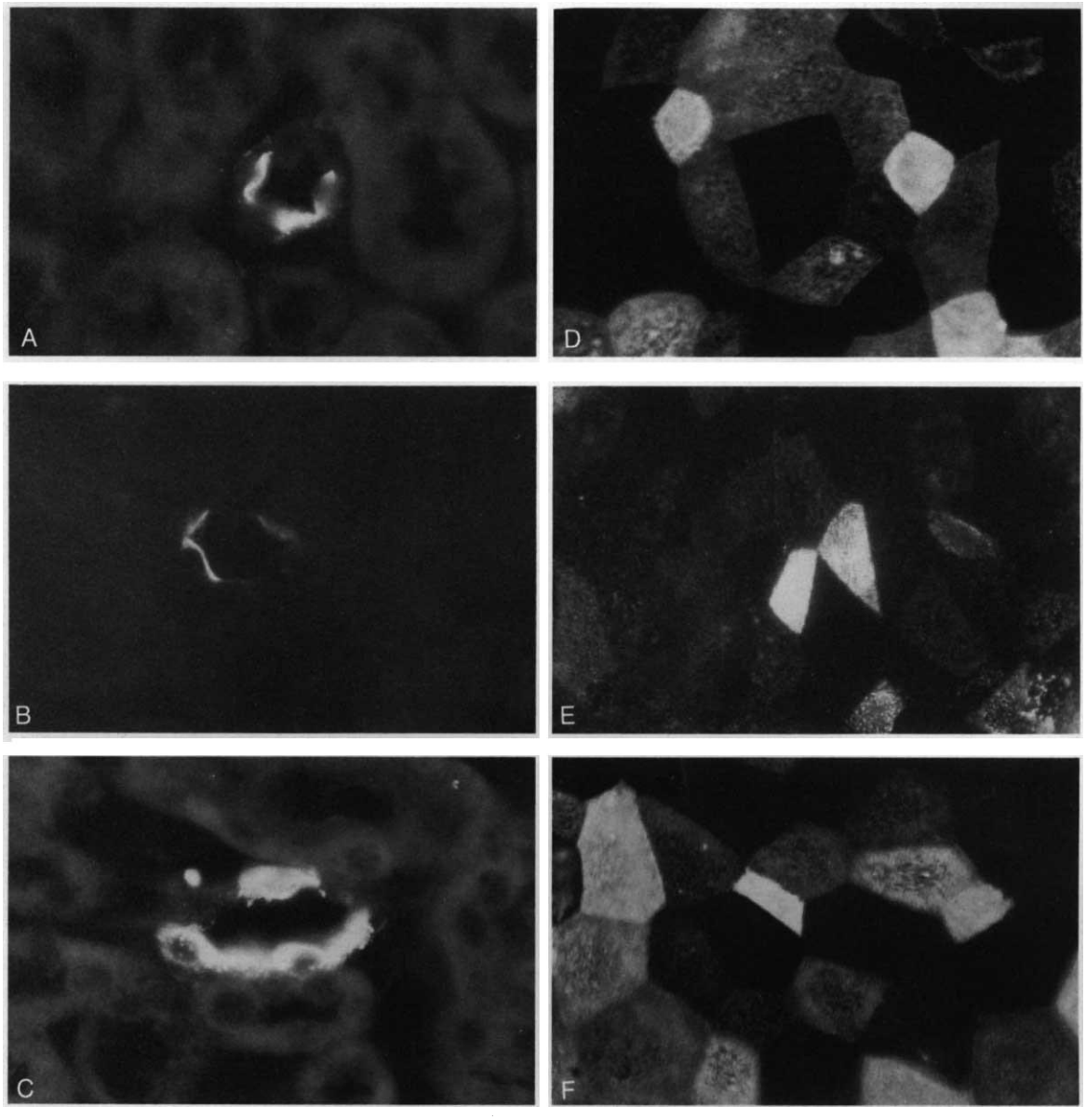

Fig. 1. *Reactivity of monoclonal antibodies with mouse kidney and M-1 cells (magnification: 540 ×).* A-C: Fluorescence photomicrographs of longitudinal sections of renal cortex stained with MCAB ST12 (**A**), MCAB B63 (**B**), and MCAB F_{13}/0121 (**C**). D-F: Fluorescence photomicrograph of M-1 cells stained with MCAB ST.12 (**D**), MCAB B63 (**E**) and MCAB F13/0121 (**F**).

M-3) continued to have an epithelium-like appearance but exhibited somewhat lower voltages and SCCs; therefore, we focused on the M-1 line.

Immunocytochemistry

Figure 1 illustrates the reactivity of the three MCABs on mouse kidney and M-1 cells. Fluorescent photomicrographs of sections of renal cortex (left hand side) and cultured cells (right hand side) correspondingly stained with MCABs: ST.12 (A,D), B63 (B,E), and F_{13}/0121 (C,F), are depicted. ST.12 recognizes an ectoantigen in mouse CCDs and predominantly stains principal cells. B63 stains only B-type intercalated cells [13]. F_{13}/0121 reacts exclusively with principal cells. Based on the reactivity with F_{13}/0121, ~75% of the cells could be categorized as principal cells. This percentage did not change with the number of passages (current passage number is 32). The three MCABs combined stained more than 95% of M-1 cells. The cultured cells did not react with antibodies against band three related antigens and did not stain for either alkaline phosphatase activity or Tamm-Horsfall protein.

Microscopy

The characteristic epithelial appearance of a confluent monolayer of M-1 cells is illustrated in Figure 2A, while Figure 2B shows a scanning electron micrograph of the cell's apical surface populated by short microvilli. The boundaries between adjacent cells are distinct; however, no cilia are apparent.

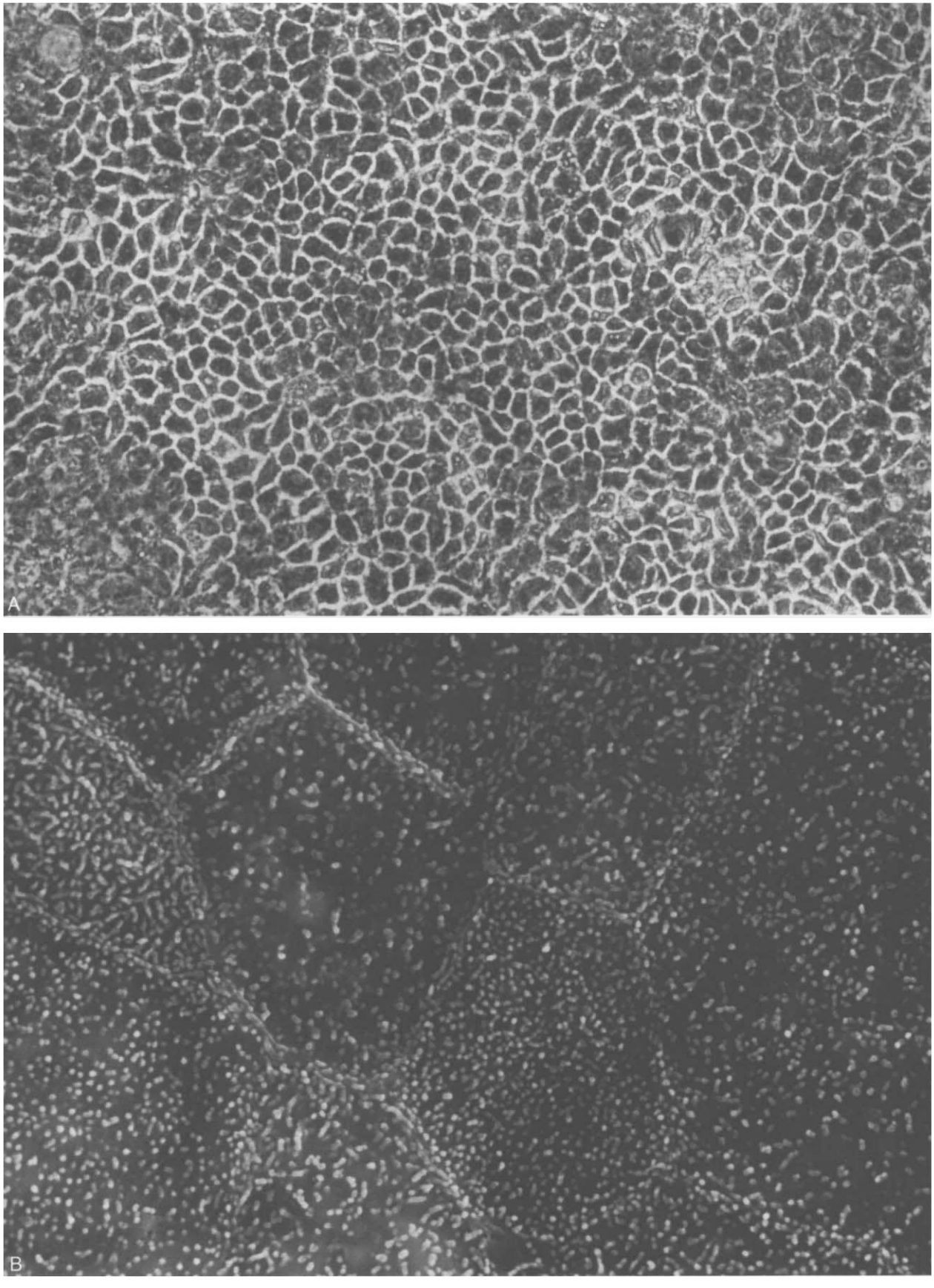

Fig. 2. A. *M-1 cells grown to confluence on a Cellagen unit (magnification: 225 ×).* **B.** Scanning electron micrograph of the apical surface (magnification: 6650 ×)

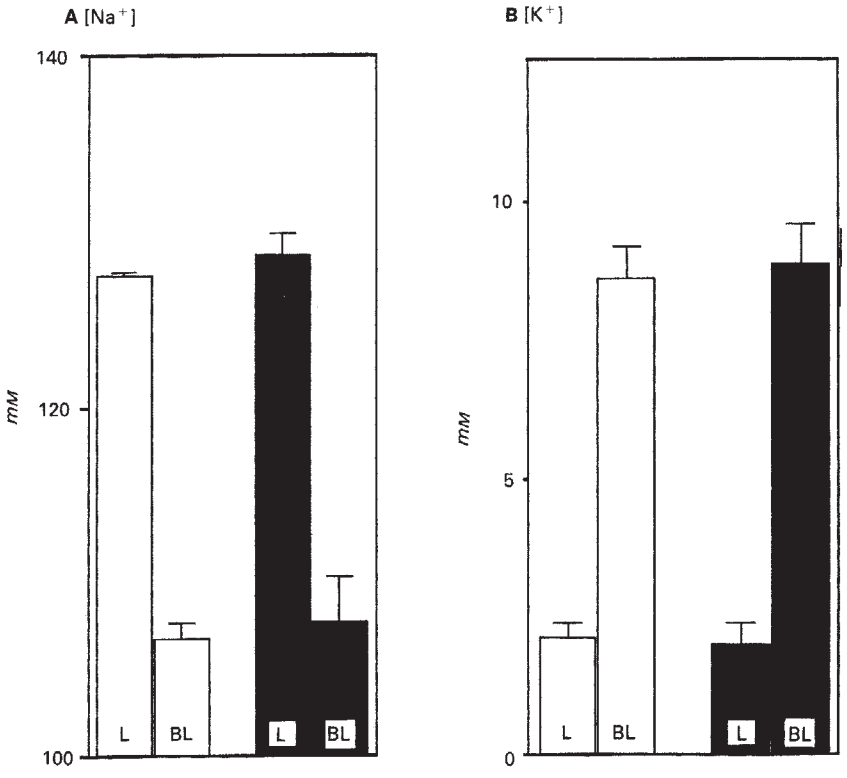

Fig. 3. *Concentration of Na^+ and K^+ in the basolateral (BL) and luminal (L) medium after 24 hours, from M-1 cells cultured in medium B at passage # 5-6 (open columns, N = 7) and passage # 12-13 (filled columns, N = 3).*

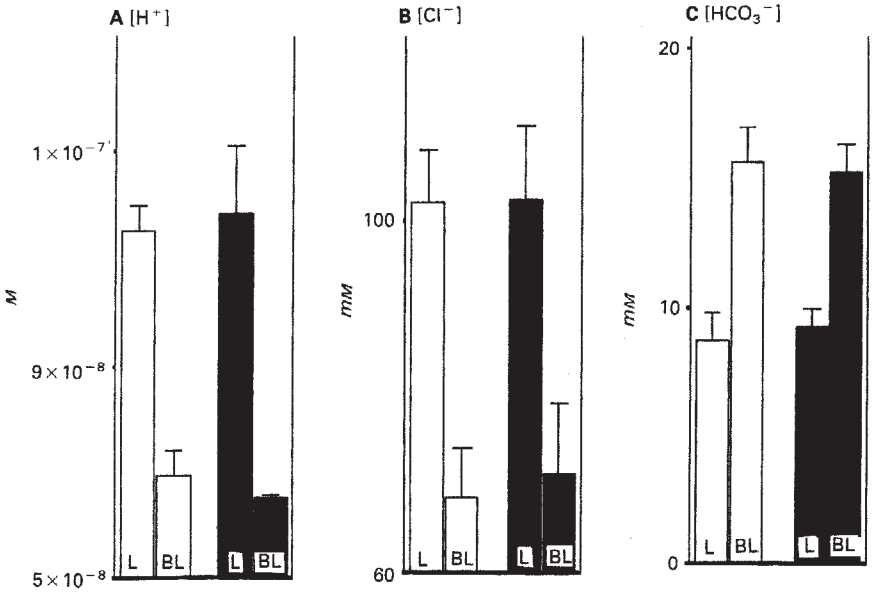

Fig. 4. *Distribution of H^+, Cl, and HCO_3^- concentrations in the basolateral and luminal medium, in M-1 cells cultured in medium B at passage # 5-6 (open columns, N = 10) and passage # 12-13 (filled columns, N = 6).*

cAMP accumulation in M-1 cells

Basal cAMP production in M-1 cell suspension was 21.8 ± 3.2 fmol/min/10^5 cells. One nanomole AVP increased cAMP accumulation to 37.4 ± 7.4 fmol/min/10^5 cells (an increase of 68.9 ± 14.9%, $P < 0.01$) while bovine PTH (10^{-6} M) had no effect (control 18.0 ± 1.6, PTH 18.6 ± 1.2 fmol/10^5 cells).

Ion transport in M-1 monolayers

The compositions of the luminal and basolateral compartments after 24 hours of incubation in medium B are shown in Figures 3 and 4 for M-1 cells at passage numbers 5-6 (open columns) and 12-13 (filled columns). In either case, Na^+ concentrations were significantly higher in the basolateral medium,

Table 1. Baseline electrical properties of M-1 cells from passage 5-6 cultured in medium A or B

	Medium A $N = 12$	Medium B $N = 10$
PD *mV*	−21.5 ± 1.6	−45.7 ± 3.5
SCC $\mu A/cm^2$	27.6 ± 3.05	71.8 ± 10.3
R *ohms/cm²*	885.7 ± 109.6	753.4 ± 102.2

while K^+ concentrations were significantly higher in the luminal medium ($P < 0.001$), suggesting that M-1 cells reabsorb Na^+ and secrete K^+. At passage number 5-6, the average basolateral-to-luminal ratios (B/L) for Na^+ and K^+ were 1.08 ± 0.01 and 0.71 ± 0.05, respectively, for cells grown in medium A; and 1.19 ± .01 and 0.27 ± 0.05 for cells maintained in medium B. On Figure 4 H^+, HCO_3^-, and Cl concentrations are shown after incubating M-1 cells from different passages in medium B for 24 hours. Cells from passage 5-6 developed a HCO_3^- concentration difference of 6.9 mM (higher on the luminal side; $P < 0.005$), which was accompanied by a Cl concentration difference of 33.2 mM in the opposite direction ($P < 0.05$). B/L ratios for HCO_3^- and Cl were 0.80 ± 0.12 and 1.17 ± 0.05, respectively, in medium A, and 0.58 ± 0.09 and 1.52 ± 0.08, respectively, in medium B. The B/L ratios for H^+ concentrations after 24 hours incubation were 1.67 ± 0.03 in medium A and 1.75 ± 0.08 in medium B. Cell monolayers initiated at passage 12-13 developed concentration ratios for HCO_3^-, Cl and H^+ similar to those developed by cells at passage 5-6 (Fig. 4). The ability to achieve similar ion concentration gradients is maintained at even higher passage numbers (currently 32).

Similarly, as in primary cultures of rabbit CCD cells [12], lactate accumulated predominantly on the basolateral side. Lactate concentrations were 7.1 ± 0.44 and 17.5 ± 0.52 mM at the luminal and basolateral sides. B/L ratios were 2.59 ± 0.13 for medium A and 2.57 ± 0.2 for medium B.

There were no measurable volume changes during this period (data not presented); however, osmolality was significantly lower in the luminal compartment (in medium A, L = 312 ± 3, B = 319 ± 2 mOsmol/kg H_2O, $P < 0.02$; in medium B, L = 272 ± 3, B = 290 ± 7 mOsmol/kg H_2O, $P < 0.005$).

Electrophysiology of M-1 cells

M-1 cells in both medium A and B developed a significant lumen-negative PD and had a high SCC, albeit the values of these parameters were different for cells maintained in the two media. Baseline values for cultures from passage 5-6 are listed in Table 1. For cells grown in medium B, PD and SCC were more than two-fold higher than for cells maintained in medium A ($P < 0.001$ for both PD and SCC). Transepithelial electrical resistance (R) of cultures in medium B tended to be lower than in medium A without reaching statistical significance. The electrophysiological parameters of cultures with higher passage numbers were very similar to those obtained with lower passage numbers. Cultures at passages 12-14, grown in medium B, had a transepithelial PD of 41.9 ± 7 mV, SCC of 71.8 ± 0.6 $\mu A/cm^2$ and resistance of 646 ± 124 ohms/cm².

Simultaneous determination of SCC and the L-to-B flux of ^{22}Na on seven monolayers revealed a close correlation between these two parameters ($r = 0.899$; $P < 0.01$). SCC was 43.5 ± 3.4 nmol/cm² while L-to-B flux of ^{22}Na was 51.7 ± 5.8 nmol/cm²; thus 86.1 ± 4% of Na^+ flux is accountable by the SCC.

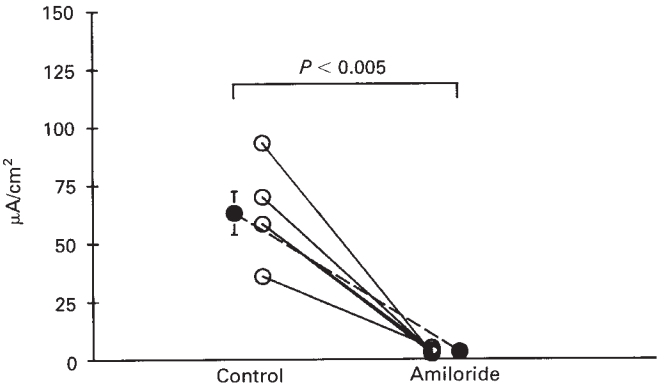

Fig. 5. *Effect of amiloride (20 μM) on SCC of M-1 cultures maintained in medium B.* Open circles and solid lines represent individual experiments, filled circles and dashed line represent the mean response.

Luminal application of 20 μM amiloride significantly reduced SCC from 27.6 ± 3.05 to 4.7 ± 1.2 $\mu A/cm^2$ ($P < 0.01$) in medium A and from 71.8 ± 10.3 to 3.4 ± 0.47 $\mu A/cm^2$ ($P < 0.005$) in medium B. Figure 5 depicts the effect of amiloride on SCC for cultures grown in medium B. The amiloride-induced changes in SCC were accompanied by a reduction in the lumen-negative PD (from −21.5 ± 1.6 to −4.9 ±1.5 mV in medium A, $P < 0.005$, and from −45.7 ± 3.5 to −5.3 ± 1.8 mV in medium B, $P < 0.005$). Amiloride increased R from 765 ± 144 to 1,465 ± 306 ohms/cm² ($P < 0.02$) in cultures in medium B, and from 872 ± 203 to 987 ± 134 (NS) in medium A.

Arginine-vasopressin (10^{-9} M) significantly increased PD from −21.5 ± 1.6 to −28.3 ± 2.9 mV ($P < 0.01$) and SCC from 27.6 ± 3.05 to 31.4 ± 3.1 $\mu A/cm^2$ ($P < 0.05$) in cells grown in medium A ($N = 7$). In cells maintained in medium B, SCC was stimulated by AVP from 71.8 ± 10.3 to 94.1 ± 20.3 $\mu A/cm^2$ ($P < 0.05$, $N = 5$). PD tended to increase and R to decrease in every experiment, however, neither of these changes reached statistical significance.

A representative experiment demonstrating the effects of AVP and amiloride on SCC is shown on Figure 6.

Discussion

Our data show that the CCD cell line described here did not lose the ability to express many critical features of the in situ phenotype. This indicates that maintenance of functional CCD cells as an established line is possible and that M-1 could prove useful as a model in studying mechanisms of ion transport in this segment.

The CCD is characterized by a high transepithelial electrical resistance and a lumen-negative transepithelial PD [20]. Such properties are demonstrated by this cell line. The main ion transport taking place in the CCD is Na^+ reabsorption via luminal Na^+ channels which can be blocked by amiloride [c.f. 21]. Active reabsorption of Na^+ in M-1 cultures was evidenced by the formation of a transepithelial Na^+ gradient against an unfavorable transepithelial PD. Our observation that under control conditions 86% of the measured lumen-to-bath ^{22}Na flux could be accounted for by the SCC suggests that Na^+ is absorbed primarily through a conductive pathway in M-1 cells.

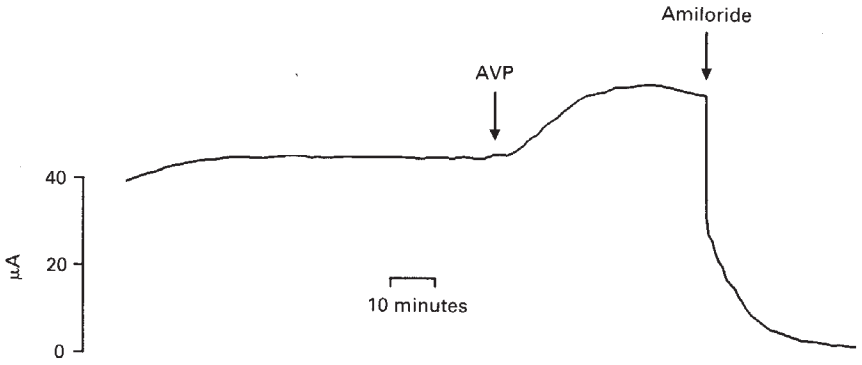

Fig. 6. *Representative tracing of the effects of AVP (1 nM) and amiloride (20 μM) on SCC in a M-1 culture maintained in medium B.*

This was confirmed by the response to amiloride which practically obliterated the SCC associated with the lumen-negative voltage. In addition to Na^+ reabsorption, the CCD secretes K^+ [22]. K^+ secretion in M-1 cells was evidence by a marked elevation in luminal K^+ concentration. Although HCO_3^- concentration was significantly higher at the luminal side at all passage numbers and in both media, it remains to be determined whether this difference represents HCO_3^- secretion or might be due to preferential accumulation of lactate at the basolateral side.

The cell line also retained another characteristics of the CCD, that is, responsiveness to AVP [20], which was evidenced both by an increase in cAMP accumulation in cell suspension and by a prompt increase in SCC following AVP administration to the basolateral side of the monolayer.

In addition to the differentiated transport functions and AVP-responsiveness, M-1 cells also retained all CCD-specific antigens studied (ST.12, B63, and F_{13}/0121). In aggregate, these data indicate that M-1 cells did not undergo a major dedifferentiation when compared to the CCD in situ.

The reason that all of these characteristics were retained by the M-1 cell line may be attributed to the fact that it originates from a transgenic animal carrying SV40 early-region genes. Differentiated cells normally do not proliferate in culture and often cease to express tissue-specific functions. This can sometimes be overcome by transfection with oncogenes. In the present study, tubules were isolated from a Tg(SV40E)Bri/7 mouse transgenic for the early region of SV40 [9, 10], in order to create a model in which the genetic background was modified to enhance cell proliferation. MacKay et al [23] used such mice to establish lines of glomerular epithelial, mesangial, and endothelial cells which maintained features characteristic of their normal counterparts. The reason why cell lines originating from Tg(SV40E)Bri/7 transgenic animals seem to retain differentiated functions so well is not clear. One possible explanation may be that a selection pressure exists in vivo that would not occur when introducing transforming genes in vitro. With in vitro transformation, differentiated functions which may not be needed for survival in culture are likely to be lost, whereas such functions could be essential for survival of the entire organism and therefore must be retained in the transgenic animal. Therefore, cell lines derived from Tg(SV40E)Bri/7 mice may be more likely to retain the characteristics of the in vivo epithelium than those initiated by introducing the same gene in vitro. In support of this idea is the report by Arend et al [8] who infected primary cultures of rabbit CCD cells with an adenovirus 12/SV40 hybrid, resulting in a cell line which retained morphological as well as some functional properties of the CCD; however, transepithelial resistance was low. Similarly, we have infected cells from rat and rabbit CCDs with SV40-tsA58 in vitro but the resulting cultures did not develop a high electrical resistance. Thus in terms of obtaining cultures which more closely express the normal cell phenotype, in vitro immortalization does not appear to be as successful as initiating cell lines from Tg(SV40E)Bri/7 transgenic mice.

In summary, M-1 is a mammalian cell line which retains many antigenic and differentiated transport properties of the CCD epithelium and thus should prove valuable as a model for study of ion transport in this nephron segment.

Acknowledgments

We are grateful to Dr. Ralph L. Brinster (University of Pennsylvania) for providing the transgenic mice, and to Drs. Victor L. Schuster (A. Einstein College of Medicine) and Seth L. Alper (Beth Israel Hospital) for providing the antibodies against band 3. This work was supported by NIH grants HL 28982 and DK 39523 and a Grant-in-Aid from the American Heart Association (89-985).

Reprint requests to G. Fejes-Tóth, Department of Physiology, Dartmouth Medical School, Hanover, New Hampshire 03756, USA.

References

1. Gstraunthaler GJA: Epithelial cells in tissue culture. *Renal Physiol Biochem* 11:1–42, 1988
2. Perkins FM, Handler JS: Transport properties of toad kidney epithelia in culture. *Am J Physiol* 241:C154–C159, 1981
3. Handler JS, Steele RE, Sahib MK, Wade JB, Preston AS, Lawson NL, Johnson JP: Toad urinary bladder epithelial cells in culture: maintenance of epithelial structure, sodium transport, and response to hormones. *Proc Natl Acad Sci USA* 76:4151–4155, 1979
4. Madin SH, Darby NB Jr: *Registry of Animal Cell Lines.* 1965 US Dept. HEW, PHS
5. Gausch CR, Hard WL, Smith TF: Characterization of an established line of canine kidney cells (MDCK). *Proc Soc Exp Biol Med* 122:931–935, 1966
6. Hull RN, Cherry R, Weaver GW: The origin and characteristics of a pig kidney cell strain LLCPK. *In Vitro* 12:670–677, 1976
7. Valentich JD, Stokols MF: An established cell line from mouse kidney medullary thick ascending limb II. Transepithelial electrophysiology. *Am J Physiol* 251:C312–C322, 1986
8. Arend LJ, Handler JS, Rhim JS, Gusovsky F, Spielman WS: Adenosine-sensitive phosphoinositide turnover in a newly established renal cell line. *Am J Physiol* 256:F1067–F1074, 1989

9. PALMITER RD, CHEN HY, MESSING A, BRINSTER RL: SV 40 enhancer and large T antigen are instrumental in development of chorioid plexus tumors in transgenic mice. *Nature* 316:457–460, 1985
10. MESSING A, CHEN HY, PALMITER RD, BRINSTER RL: Peripheral neuropathies, hepatocellular carcinomas and islet cell adenomas in transgenic mice. *Nature* 316:461–463, 1985
11. MOREL F, CHABARDES D, IMBERT M: Methods in pharmacology, in *Renal Pharmacology*, edited by MARTINEZ-MALDONADO M, New York, Plenum Press, 1976, pp. 297–323
12. FEJES-TÓTH G, NÁRAY-FEJES-TÓTH A: Differentiated transport functions in primary cultures of rabbit collecting ducts. *Am J Physiol* 253:F1302–F1307, 1987
13. FEJES-TÓTH G, MEHRGUT FM, NÁRAY-FEJES-TÓTH A, SCHWARTZ GJ: Monoclonal antibody (MCAB) against a luminal antigen on B-intercalated cells (B-ICC) inhibits HCO_3 secretion. (abstract) *Kidney Int* 36:536, 1990
14. HOYER JR, SEILER MW: Pathophysiology of Tamm-Horsfall protein. *Kidney Int* 16:279–289 1979
15. SCHUSTER VL, BONSIB SM, JENNINGS ML: Two types of collecting duct mitochondria-rich (intercalated) cells: Lectin and band 3 cytochemistry. *Am J Physiol* 251:C347–C355, 1986
16. ALPER SL, NATALE J, GLUCK S, LODISH HF, BROWN D: Subtypes of intercalated cells in rat kidney collecting duct defined by antibodies against erythrocyte band 3 and renal vacuolar H-ATPase. *Proc Natl Acad Sci USA* 86:5429–5433, 1989
17. FORRESTER RL, WATAJI LJ, SILVERMAN DA, PIERRE KJ: Enzymatic method for determination of CO_2 in serum. *Clin Chem* 22:243–245, 1976
18. FEJES-TÓTH G, NÁRAY-FEJES-TÓTH A: Isolated principal and intercalated cells: hormone responsiveness and Na^+-K-ATPase activity. *Am J Physiol* 256:F742–F750, 1989
19. NÁRAY-FEJES-TÓTH A, FEJES-TÓTH G: Glucocorticoid receptors mediate mineralocorticoid-like effects in cultured collecting duct cells. *Am J Physiol* 259:F672–F678, 1990
20. BURG MB, ISAACSON L, GRANTHAM J, ORLOFF J: Electrical properties of isolated perfused rabbit renal tubules. *Am J Physiol* 215:788–794, 1968
21. SARIBAN-SOHRABY S, BENOS DJ: The amiloride sensitive Na channel. *Am J Physiol* 250:C175–C190, 1986
22. GIEBISCH G: Renal potassium transport, in *Membrane Transport in Biology*, edited by GIEBISCH G, TOSTESON D, USSING HH, Berlin, Heidelberg, New York, Springer Verlag, Vol IVA, 1978, p. 215
23. MACKAY K, STRIKER LJ, PINKERT CA, BRINSTER RL, STRIKER GE: Glomerulosclerosis and renal cysts in mice transgenic for the early region of SV40. *Kidney Int* 32:827–837, 1987