American Journal of Pathology, Vol. 147, No. 5, November 1995

Chronic Systemic Treatment with Epidermal Growth Factor in Pigs Causes Pronounced Urothelial Growth with Accumulation of Glycoconjugates

Lars Vinter-Jensen,*† Claus Orloff Juhl,† Jens C. Djurhuus,† Steen Seier Poulsen,‡ Esam Z. Dajani,§ Kenneth D. Brown Ph. D.,‖ Torben F. Ørntoft,* Peter Stubbe Teglbjærg,¶ and Ebba Nexø*

From the Department of Clinical Biochemistry, Aarhus University Hospital and Institute of Experimental Clinical Research,† University of Aarhus, Aarhus, Denmark; Institute of Medical Anatomy,‡ The Panum Institute, University of Copenhagen, Copenhagen, Denmark; International Drug Development Consultants,§ a Division of Mid-Gulf USA, Inc., Long Grove, Illinois; The Babraham Institute,‖ Cambridge, United Kingdom; and Institute of Pathology,¶ Aalborg Hospital, Aalborg, Denmark*

Epidermal growth factor (EGF) is present in large amounts in the urine, but the effects of systemically administered EGF on the urinary tract have not been described previously. In the present paper, we describe a potent growth induction of EGF on the urinary tract. Goettingen minipigs were treated with solvent (n = 5), EGF 30 μg/kg/day (n = 6) for 4 weeks, or EGF 30 μg/kg/day for 5 weeks followed by 3 weeks of recovery (n = 5). The ureters and bladders were examined by routine histology and electron microscopy and were immunostained for proliferating cell nuclear antigen. Four weeks of EGF treatment increased the median cross sectional area of the ureter fourfold with growth of all wall layers. The urothelium was widened from 5 cell layers in the controls to 10 in the EGF-treated animals. Proliferating cell nuclear antigen immunostaining revealed an increased mitotic activity in the basal zone of the urothelium. In the luminal zone, glycoconjugates accumulated in goblet cells, in cells with intracytoplasmic lumina, and beneath the luminal cell membrane in the umbrella cells. Our studies present a new experimental approach to growth induction of the urinary tract. The findings implicate the EGF system in regulating urothelial growth and glycoconjugate biosynthesis. (Am J Pathol 1995, 147:1330–1338)

The epidermal growth factor (EGF) system is of importance in normal, regenerative and neoplastic growth.[1] EGF and related ligands such as transforming growth factor-α (TGF-α) exert their effects through binding to the same EGF receptor.[1] The action of the ligands depends on the context in which they operate; ie, when administered around the time of birth EGF accelerates eyelid opening[2] and lung maturation[3] (for review see Carpenter and Wahl[4]), whereas transgenic mice overexpressing TGF-α develop epithelial changes in several organs, mainly the pancreas, liver, and mammary glands.[5–7]

EGF is synthesized in the kidneys and secreted in an exocrine manner to the urine.[8,9] Contact of urinary EGF with the urothelium may exert a trophic action on this tissue.[10] Few studies have been performed on the distribution of the EGF family within the wall of the normal urinary tract; TGF-α and the EGF receptor have been described within the urothelium.[10,11] In contrast several studies have examined the EGF growth factor system in the urothelium in relation to malignancy (for review see Neal and Mellon[12]).

The systemic effects of EGF and its therapeutic potential for stimulating tissue repair have been explored in relation to different organs.[13–15] We investigated treatment effects of EGF on experimentally induced damage of the esophagus of Goettingen minipigs.[13,16] In the course of this work, we discovered several unknown effects of EGF. The most pro-

Supported in part by the Danish Medical Research Council, 12–1317 by The Institute of Experimental Clinical Research, University of Aarhus and by Gastrone Inc., Menlo Park, CA.

Accepted for publication July 13, 1995.

Address reprint requests to Lars Vinter-Jensen, MD, Department of Clinical Biochemistry, KH, Aarhus University Hospital, Nørrebrogade 44, DK-8000 Aarhus C, Denmark.

nounced findings were considerably enlarged ureters and epithelial hyperplasias with intracellular glycoconjugate accumulations in the urothelium and excretory ducts of the pancreas. In the present paper, we describe the potent growth-promoting effect on all layers of the ureters with focus on the hyperplastic urothelium.

Materials and Methods

Study Animals

The study comprised 11 Goettingen minipigs, of both sexes, weighing 17.5 to 23.4 kg (ranges), ~12 months of age. The animals were housed individually in pigsties. Once a week they were placed in a metabolic cage for urine sampling. They were allowed free access to water, which was never more than 4 L. The protocol complied with Danish and United States regulations for the care and use of laboratory animals.

Design and Treatment

A detailed description of the study is given in Juhl et al.[16] In brief, portal hypertension was induced by banding of the portal vein as previously described.[17] The animals were allowed to recover for 6 weeks. They underwent, thereafter, a period of 4 weeks with a weekly endoscopic sclerotherapy session, daily treatment with EGF or placebo, and they were subsequently sacrificed at the end of week 4.

All animals received subcutaneous injections of human recombinant EGF (lot no. 11435, Upstate Biotechnology, NY) or vehicle (Tris-HCl) during the 4 weeks as detailed below:

Group 1, Controls (n = 5)

Vehicle (Tris-HCl) was administered subcutaneously three times daily for 28 days.

Group 2, EGF Treatment (n = 6)

EGF (10 μg/kg) was administered subcutaneously three times daily for 28 days.

Group 3, EGF Treatment and Recovery (n = 5)

To evaluate the morphology of the ureter after recovery from EGF treatment, tissue samples were obtained from five additional Goettingen minipigs from another similar treatment series.[13] These animals were injected three times daily with EGF (10 μg/kg) for 5 weeks and were given an additional intraesophageal intramural injection of EGF (20 μg/kg) once a week. They recovered, thereafter, without EGF treatment, for 3 weeks before sacrifice.

One animal from the control group died one week before the planned termination because of a defective respirator. Tissue samples for histological analysis were obtained from this animal immediately after its death.

Autopsy and Preparation of Tissue Samples

At sacrifice, tissue samples were collected from ureters 3 cm below the left renal pelvis and from the fundus of the bladder. The samples were fixed in a 4% solution of neutrally buffered paraformaldehyde, routinely processed, and embedded in paraffin. Sections (5 μm) for morphological examination were stained with hematoxylin and eosin (H&E), van Gieson's stain, and periodic acid-Schiff/Alcian blue at pH 2.7 (PAB) with and without diastase pretreatment.

The ureters were cut perpendicular to the longitudinal axis, and the bladder was sectioned perpendicular to the luminal surface. The number of cell layers of the urothelium was counted. The cross-sectional area (CSA) of the ureter was calculated as follows. The sections were evaluated using a projection microscope with projection to a test system with regularly arranged points (1 cm between each point) at a magnification of ×72.5. The number of points falling into the muscle, submucosa, epithelium, and lumen were counted and the CSAs of the respective layers calculated.

Electron Microscopy

The ureters of one placebo- and two EGF-treated animals were used for electron microscopy. After primary fixation in paraformaldehyde, small pieces of tissues were immersed in glutaraldehyde (2.5%) in 0.1 mol/L cacodylate buffer (pH 7.3), postfixed for 1 hour in 1% osmium tetroxide in the same buffer, dehydrated in alcohol and propylene oxide, and embedded in Epon 812. The ultrathin sections were stained with uranyl acetate and lead citrate.

Immunohistochemistry for Proliferating Cell Nuclear Antigen (PCNA)

PCNA, a highly conserved DNA polymerase-δ-associated protein, was used as a marker for cell proliferation.[18] Sections from the ureters (from all animals

from groups 1 and 2) were stained with a mouse monoclonal antibody against PCNA (PC 10, lot no. 121, DAKO, Copenhagen, Denmark). Five μm thick, paraformaldehyde-fixed, paraffin-embedded sections were employed. The sections were treated in jars with sterile demineralized water for 10 minutes in a household microwave oven (Miele Electronic M696, Germany) at 700 W and incubated overnight with the primary antibody against PCNA (diluted 1:20). The second layer was biotinylated rabbit anti-mouse immunoglobulin (DAKO), diluted (1:400) for 30 minutes, followed by incubation with the horseradish peroxidase-labeled streptavidin (DAKO, Copenhagen) according to the manufacturer's instructions. Color development was done with 3-amino-9-ethyl-carbazole, and cell nuclei were stained using Mayer's hematoxylin. Because of an obvious difference in number of immunostained nuclei in the controls and EGF-treated animals, only a gross histological description is given and illustrated.

Immunohistochemistry for Human EGF

In group 2, human recombinant EGF was administered subcutaneously 8 hours before sacrifice. We have previously demonstrated that human recombinant EGF is detectable in plasma up to 12 hours after subcutaneous administration in pigs.[13] To investigate the tissue binding of the human recombinant EGF, paraformaldehyde-fixed, paraffin-embedded sections were stained with an EGF antibody on two placebo and two EGF-treated animals. The sections were pretreated with 10% porcine serum for 30 minutes and incubated overnight with antisera against EGF (4554 and 90001) diluted 1:400 and 1:1600. The immunoreaction was visualized by the unlabeled peroxidase-antiperoxidase technique using porcine antirabbit IgG (Z196, Dakopatt, Copenhagen, Denmark) and peroxidase-antiperoxidase (Z113, Dakopatt) and diaminobenzidine for staining. For control the primary antisera were preincubated with excess of antigen (30 μg/ml).

Measurements of Human and Pig EGF in Urine

The urinary concentration of EGF was evaluated by measuring human recombinant as well as pig EGF in urine obtained once per week from each animal. Human EGF was measured as previously described.[19] Pig EGF was quantitated by an enzyme-linked immunosorbent assay method developed as previously described for rat and human EGF.[19,20] Recombinant pig EGF was employed as calibrator (0.04 to 0.25 nmol/L), and the γ-globulin fraction of a polyclonal rabbit antiserum raised against recombinant pig EGF[21] was employed as catching and detecting antibody. Human EGF of 1 nmol/l was recognized as <0.04 nmol/L.

Statistics

Data are given as median and ranges. Mann-Whitney's nonparametric test for nonpaired data was employed. The level of significance chosen was 0.05.

Results

The animals thrived throughout the study with no weight differences between the groups. At autopsy, the ureters appeared thicker and longer in the EGF-treated animals.

Microscopic Examination

Gross histological examination revealed ureters increased approximately fourfold in CSA because of enlargement of all components of the ureter wall (Figure 1, Table 1). After 3 weeks of recovery from EGF treatment in group 3, the CSA decreased but remained greater than control values.

The urothelium appeared hyperplastic with an increase in cell layers from 4 to 5 to ~10 after 4 weeks of EGF treatment. The urothelium returned to a thickness of four to five cell layers after 3 weeks of recovery (Figure 2). Based on the PAB staining with and without diastase pretreatment, the control urothelium was divided principally into three zones: a basal zone of one cell layer with unstained cells; an intermediate zone of three cell layers where the PAB staining appeared as small magenta colored glycogen granules, which could be digested with diastase; and a luminal zone consisting of umbrella cells, which stained with PAB with and without diastase pretreatment. In the EGF-induced hyperplastic urothelium, the basal zone was widened to three to four layers of cells in most animals. The intermediate zone characterized by glycogen granules was also widened. The luminal zone was expanded to include the luminal two to three and four to five cell layers in the bladder and ureter, respectively. This zone was characterized by accumulation of PAB-positive material in colors from magenta to deep blue in an increased number of goblet cells, in cells with intracytoplasmic lumina, and below the luminal cell membrane of the umbrella cells. These changes

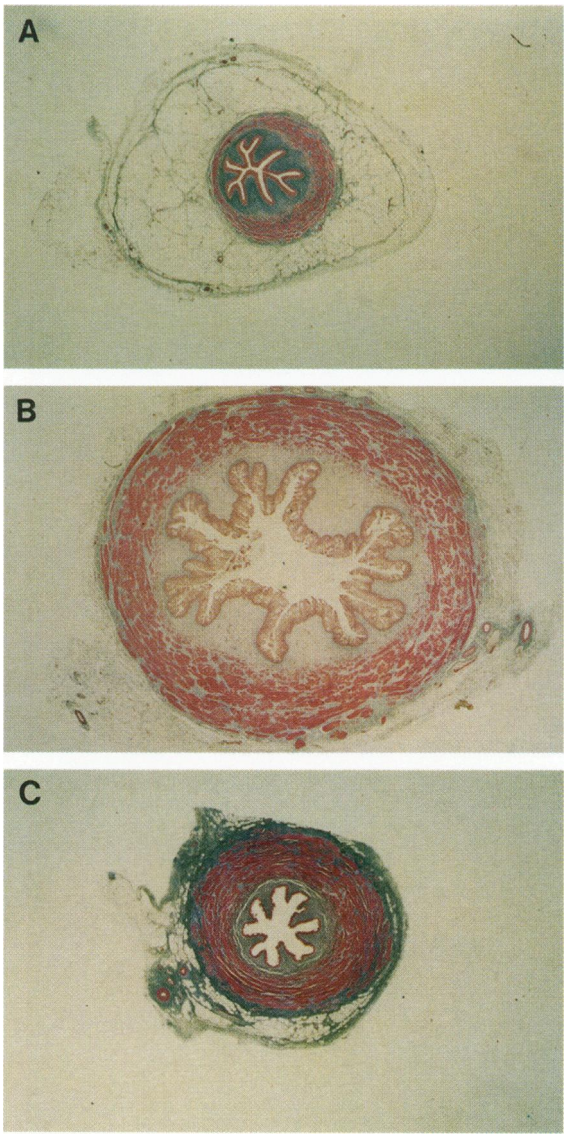

Figure 1. *Ureters from Goettingen minipigs treated with placebo for 4 weeks* (**A**), *EGF (30 μg/kg/day) for 4 weeks* (**B**), *or EGF (30 μg/kg/day) for 5 weeks followed by 3 weeks of recovery* (**C**). *The sections are stained with MDS Trichrome. (Magnification ×8).*

were more pronounced in the ureter than in the bladder. After 3 weeks of recovery from treatment, the urothelium had returned to its normal thickness and CSA. Goblet cells and intracytoplasmic lumina were still observed, and the latter structures were at this time always located just beneath the lumen.

Electronmicroscopic Examination

The morphology of the control urothelium is demonstrated in Figure 3. Single cells resembling mucinous goblet cells were observed beneath the umbrella cells. The most remarkable changes in the EGF-induced hyperplastic urothelium were seen in the luminal half of the urothelium corresponding to the luminal zone on the light microscopic examination (Figure 4). A large number of goblet cells and cells with intracytoplasmic lumina of various sizes were present. In the goblet cells mucin granules enveloped by a plasmalemma membrane were visible. The intracytoplasmic lumina were bordered by the cellular membrane from which microvilli projected into the lumina. The glycoconjugates in the intracytoplasmic lumina appeared as fibrogranular threads. Also in the luminal parts of the umbrella cells an increased amount of secretory vesicles was demonstrated.

Immunohistochemistry for PCNA

In the control urothelium PCNA staining was demonstrated in scattered cells in the basal cell layer (Figure 5A). In addition, very few nuclei in the intermediate zone and in the umbrella cell layer stained. In the EGF-induced hyperplastic urothelium the interindividual variation was more pronounced than in the controls. The number of nuclei with PCNA immunoreactivity was considerably increased, especially in the basal cell layer (Figure 5B). The cells lying in the next layers stained with gradually declining intensity, demonstrating that the cells moved upward after division. Scattered nuclei stained heavily in the intermediate and luminal zones.

Immunohistochemistry for Human EGF

In the EGF-treated animals, EGF immunoreactivity was visualized on several cells in the basal cell layer of the

Table 1. *CSA of Ureters from Treated Goettingen Minipigs*

Group	Treatment	Ureter	Muscle	Submucosa	Urothelium	Lumen
1	Placebo (n = 5)*	4.6 (3.2 to 6.4)	2.8 (1.8 to 3.6)	0.8 (0.5 to 1.5)	0.6 (0.4 to 0.9)	0.2 (0.1 to 0.4)
2	EGF (n = 6)†	17.2 (10.0 to 43.8)	12.0 (6.8 to 27.5)	2.7 (1.7 to 12.9)	2.1 (1.1 to 7.5)	0.5 (0.5 to 3.9)
3	EGF (n = 5)‡	12.7 (10.9 to 13.2)	10.1 (8.6 to 12.7)	1.8 (1.3 to 2.4)	0.5 (0.3 to 0.9)	0.6 (0.1 to 0.8)

Values are in mm^2 and given as median (range).
*4 weeks of treatment.
†4 weeks of treatment, 30 μg/kg/day.
‡5 weeks of treatment, 30 μg/kg/day, 3 weeks of recovery.

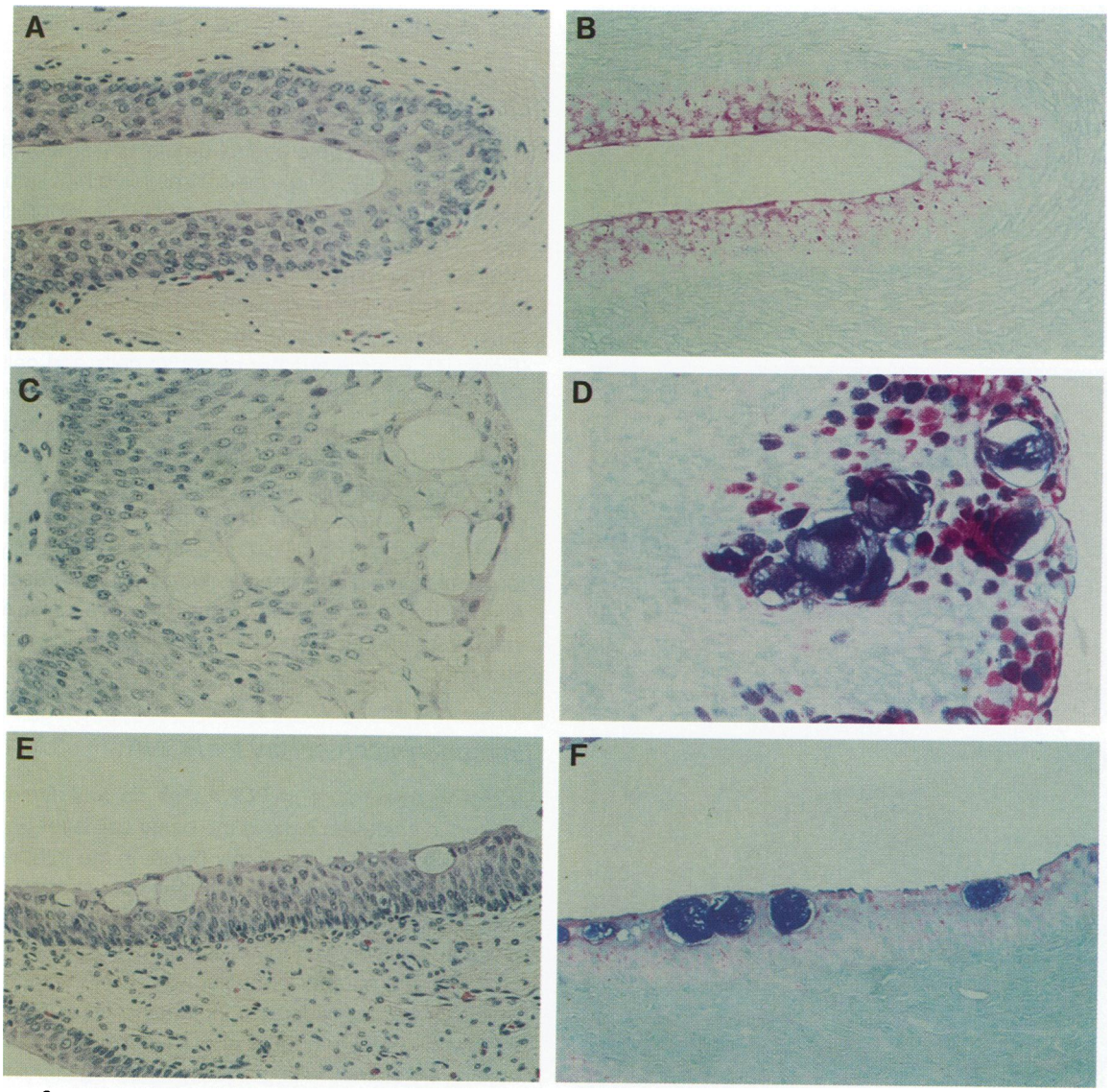

Figure 2. *Ureteric urothelium from Goettingen minipigs treated with placebo for 4 weeks* (**A**, **B**), *EGF (30 μg/kg/day) for 4 weeks* (**C**, **D**), *or EGF (30 μg/kg/day) for 5 weeks followed by 3 weeks of recovery* (**E**, **F**). *Sections* **A**, **C**, *and* **E** *are stained with H&E. Sections* **B**, **D**, *and* **F** *are stained with PAB at pH 2.7. (Magnification ×165).*

urothelium (Figure 6). The immunoreaction was inhibited by preincubation of the antibody with human recombinant EGF. No immunoreactivity for human EGF was seen in the placebo animals examined.

Excretion of Pig and Human EGF

The concentration of pig EGF in urine did not differ between the groups before the systemic treatment with human recombinant EGF began. The concentration of pig EGF in the urine of the EGF-treated animals was significantly reduced after 1 week of treatment ($P < 0.02$) and stayed at the same reduced level throughout the treatment period ($P < 0.02$ for the comparison each week). The figures for all measurements in the placebo- and EGF-treated groups were 6.8 (range 1.4 to 13.0) nmol/L and 1.8 (range 0.4 to 4.7) nmol/L, respectively. The concentration of human EGF in the urine of the treated animals ranged from 0.0 to 0.5 nmol/l. Diuresis was similar in the placebo- and EGF-treated groups based on the measurements of water intake. A total urinary EGF overload in the EGF-treated animals was thereby excluded.

Discussion

The pronounced growth of the urinary tract due to EGF treatment was an unexpected response. Al-

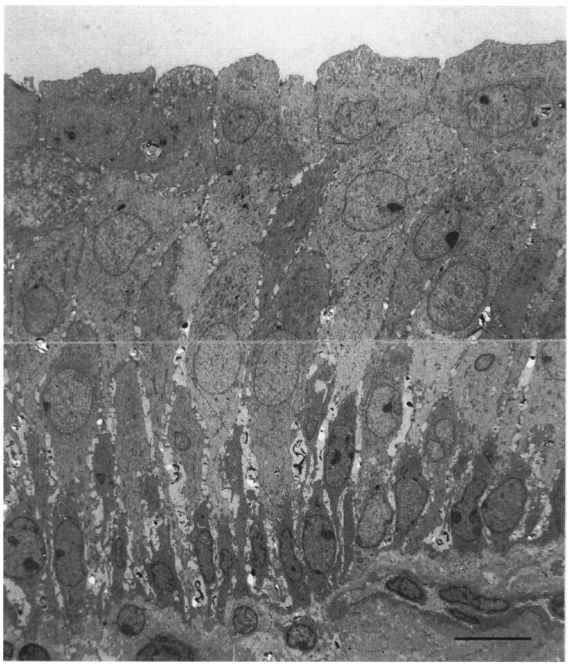

Figure 3. *Electron micrograph of ureteric urothelium from Goettingen minipig treated for 4 weeks with placebo. In the upper left corner below the umbrella cell layer a single goblet cell is visible. (Magnification ×1000; bar indicates 10 μm).*

though several investigators have induced EGF receptor hyperstimulation by treating animals with EGF or by generating transgenic mice overexpressing TGF-α[5–7] and related ligands,[22] this remarkable growth effect has not been previously reported. Pathological or experimental growth of the ureters and the bladder is usually confined to conditions characterized by urinary overload, ie, obstruction or hyperdiuresis. The growth during these conditions involves mainly the muscular coat and the connective tissue.[23] In contrast, EGF treatment induces pronounced urothelial hyperplasia without noticeable urinary overload, in addition to effects on muscle and connective tissue. Our data, together with recent preliminary data demonstrating upregulated EGF receptor gene transcription proximal to an ureteric obstruction,[24] make it important to clarify whether EGF receptor hyperstimulation is part of the mechanism leading to growth of the urinary tract in urinary overload.

EGF is synthesized in the kidney, from which it is excreted into the urine.[8] It has been proposed that urinary EGF exerts a trophic action to the urothelium[10] similar to the well recognized trophic effect of salivary EGF on gastric mucosa.[25] It is therefore tempting to speculate that increased urinary load of EGF caused by filtration of the administered EGF induced the urothelial growth. However, several unambiguous findings indicate an antiluminal effect. First, in pigs only a few percent of systemically administered EGF appears in the urine.[26] Second, total urinary EGF concentration was decreased. Third, systemically administered human EGF bound to the basal urothelial cell layer of the hyperplastic urothelium. An antiluminal effect of EGF on the urothelium is consistent with observations in the stomach. EGF in gastric juice cannot inhibit gastric acid secretion,[25] whereas systemically administered EGF and TGF-α are potent inhibitors.[27] At present, we cannot explain the reason for the reduced concentrations of pig EGF in the urine of the EGF-treated animals. They may be a consequence of an inhibitory effect of exogenous EGF on the renal endogenous EGF production and/or secretion, or they may reflect some degree of renal impairment.

In contrast to the dramatic growth reported here, two recent studies found that urothelial cells in culture proliferated in an EGF-independent manner.[11,28] There is no simple explanation for these apparently contrasting findings, but growth factor requirements *in vitro* may differ from those that limit proliferation *in vivo*. In addition, cellular interactions within tissues may influence growth factor responsiveness *in vivo*.

The PCNA staining demonstrated an increased number of cell divisions in the basal proliferative zone as marked by the lack of glycogen staining with PAB. The number of cells immunostained with PCNA might overestimate the actual proliferation as PCNA expression can be upregulated in nondividing epithelial cells close to tumors or by *in vivo* administration of EGF and TGF-α.[29] In the luminal zone, major changes had taken place with accumulation of glycoconjugates in an increased number of goblet cells, in cells with intracytoplasmic lumina, and below the luminal cell membrane in the umbrella cells. These glycoconjugates represent most likely a mixture of mucins and glycosaminoglycans as judged from the electronmicroscopic appearance and the staining with PAB. It may have clinical implications that EGF stimulates urothelial glycoconjugate biosynthesis. Glycoconjugates take part in urothelial protection and are of importance in preventing urogenital infections and for the impairment of crystal adhesions initiating stone formations.[30,31]

The presence of the intracytoplasmic lumina is somewhat intriguing. During the development of these structures, vesicles normally confined to the Golgi apparatus differentiate around a lumen within a cell.[32] Intracytoplasmic lumina are rare in normal epithelia but are a common finding in malignant epithelia in various organs, including the urothelial

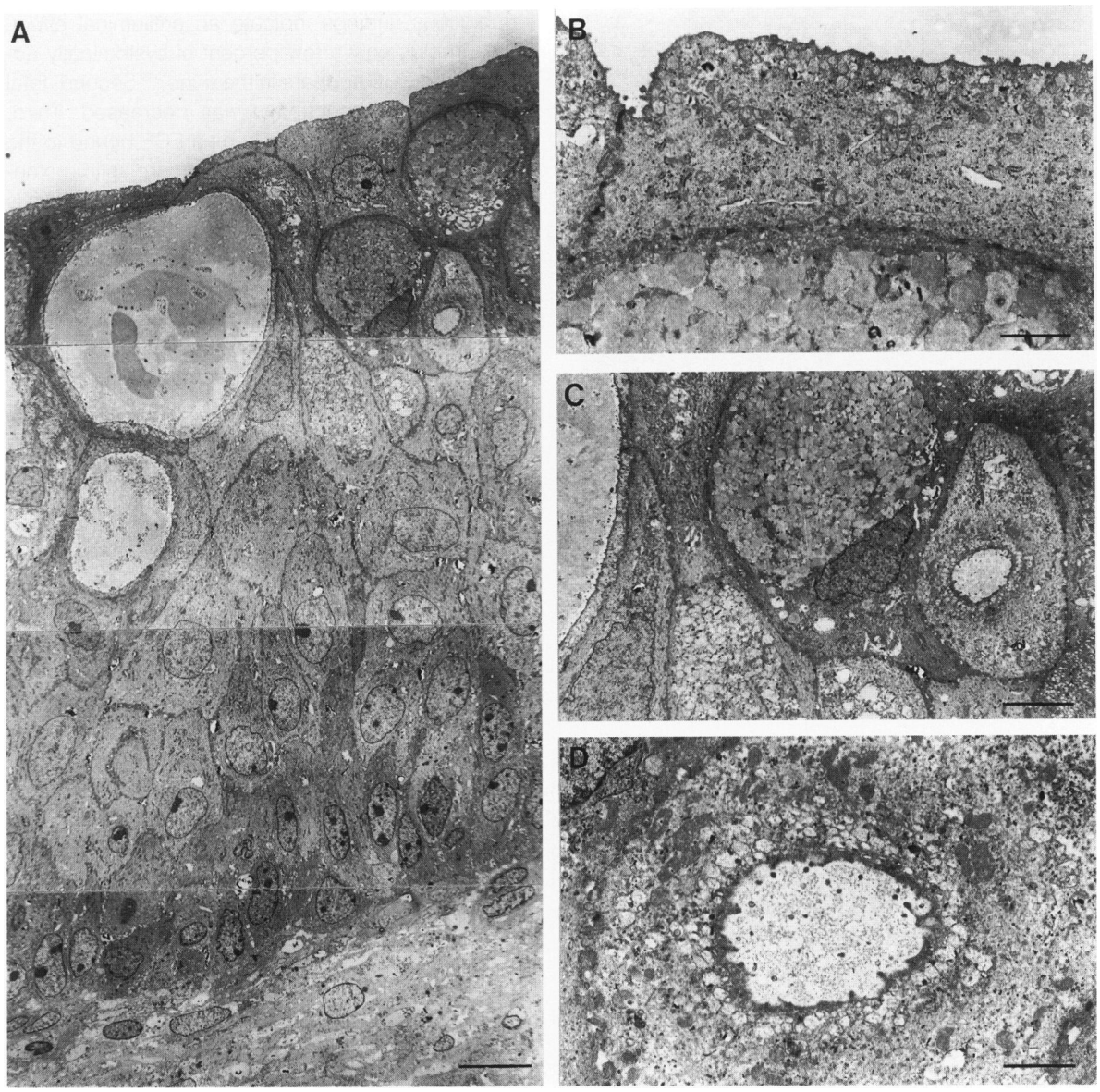

Figure 4. *Electron micrographs of ureteric urothelium from Goettingen minipig treated with EGF (30 μg/kg/day) for 4 weeks.* (**A**) *Total width of the urothelium. In the luminal half of the urothelium are several goblet cells and cells with intracytoplasmic lumina. The intracytoplasmic lumina are of various sizes. (Magnification ×1000; bar indicates 10 μm).* (**B**) *Umbrella cell and goblet cell from the upper right corner of figure 4A. (Magnification ×6667; bar indicates 1.5 μm).* (**C**) *Goblet cell and cell with a minor intracytoplasmic lumen from the upper right corner of Figure 4A. (Magnification ×2000; bar indicates 5 μm).* (**D**) *Intracytoplasmic lumen from Figure 4C. (Magnification ×6667; bar indicates 1.5 μm).*

tract.[33–35] It has been proposed that their formation is due to an alteration of the secretory system after loss of differentiation,[32] but the formation of these structures might be due solely to increased glycoconjugate biosynthesis.[32]

In conclusion, our studies describe a new experimental approach to induction of growth of the urinary tract by chronic systemic administration of EGF. The urothelial growth was reversible and characterized by increased mitotic activity in the basal zone and accumulation of glycoconjugates in goblet cells, in cells with intracytoplasmic lumina, and below the luminal cell membrane in the umbrella cells. The findings implicate the EGF system in regulating urothelial growth and glycoconjugate biosynthesis.

Acknowledgments

We wish to thank consultants Henrik Barlebo, MD, and Karsten Nielsen, MD, Dr Med Sci, of the Institute of Pathology, Aalborg Hospital for valuable discussions throughout the preparation of this manuscript.

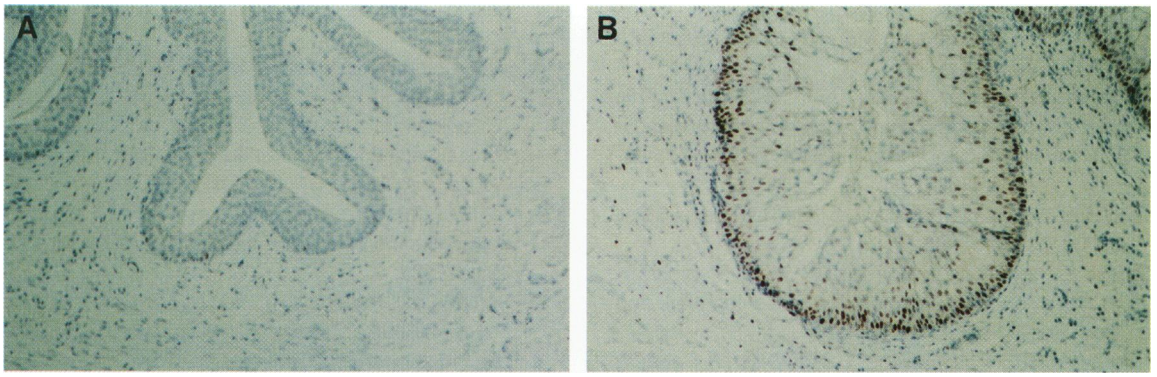

Figure 5. *Immunostaining for PCNA in ureters from Goettingen Minipigs treated for 4 weeks with placebo* (**A**) *or EGF (30 μg/kg/day)* (**B**). *(Magnification ×72).*

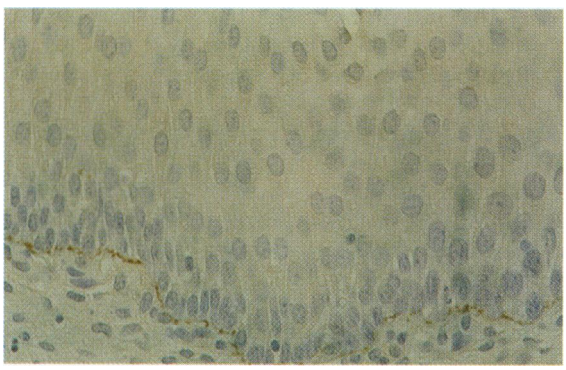

Figure 6. *Immunostaining for human recombinant EGF in a Goettingen minipig treated for 4 weeks with EGF (30 μg/kg/day). The immunostaining is seen on the basal cell layer. (Magnification ×250).*

References

1. Prigent SA, Lemoine NR: The type 1 (EGFR-related) family of growth factor receptors and their ligands. Prog Growth Factor Res 1992, 4:1–24
2. Cohen S: Isolation of a mouse submaxillary gland protein accelerating incisor eruption and eyelid opening in the newborn animal. J Biol Chem 1962, 237:1555–1562
3. Plopper CG, St. George JA, Read LC, Nishio SJ, Weir AJ, Edwards L, Tarantal AF, Pinkerton KE, Merritt TA, Whitsett JA, George-Nascimento C, Styne D: Acceleration of alveolar type II cell differentiation in fetal rhesus monkey lung by administration of EGF. Am J Physiol 1992, 262:L313–L321
4. Carpenter G, Wahl MI: The epidermal growth factor family. Handbook of Experimental Pharmacology. Peptide Growth Factors and Their Receptors I and II. Edited by MB Sporn and AB Roberts. Berlin, Springer-Verlag, 1990, pp 69–171
5. Jhappan C, Stahle C, Harkins RN, Fausto N, Schmidt GH, Merlino GT: TGFα overexpression in transgenic mice induces liver neoplasia and abnormal development of the mammary gland and pancreas. Cell 1990, 61:1137–1146
6. Sandgren EP, Luetteke NC, Palmiter RD, Brinster RL, Lee DC: Overexpression of TGFα in transgenic mice: induction of epithelial hyperplasia, pancreatic metaplasia and carcinoma of the breast. Cell 1990, 61:1121–1135
7. Matsui Y, Halter SA, Holt JT, Hogan BL, Coffey RJ: Development of mammary hyperplasia and neoplasia in MMTV-TGFα transgenic mice. Cell 1990, 61:1147–1155
8. Rall LB, Scott J, Bell GI, Crawford RJ, Penschow JD, Niall HD, Coghlan JP: Mouse prepro-epidermal growth factor synthesis by the kidney and other tissues. Nature 1985, 313:228–231
9. Jørgensen PE, Hilchey SD, Nexø E, Poulsen SS, Quilley CP: Urinary epidermal growth factor is excreted from the rat isolated perfused kidney in the absence of plasma. J Endocrinol 1993, 139:227–234
10. Messing EM, Hanson P, Ulrich P, Erturk E: Epidermal growth factor, interactions with normal and malignant urothelium: *in vivo* and *in situ* studies. J Urol 1987, 138:1329–1335
11. Cilento BG, Freeman MR, Schneck FX, Retik AB, Atala A: Phenotypic and cytogenetic characterization of human bladder urothelia expanded *in vitro*. J Urol 1994, 152:665–670
12. Neal DE, Mellon K: Epidermal growth factor receptor and bladder cancer: a review. Urol Int 1992, 48:365–371
13. Juhl CO, Vinter-Jensen L, Jensen LS, Nexø E, Djurhuus JC, Dajani EZ: Recombinant human epidermal growth factor accelerates healing of sclerotherapy-induced esophageal ulcers and prevents esophageal stricture formation in pigs. Dig Dis Sci 1994, 39:2671–2678
14. Thompson JS, Saxena SK, Greaton C, Schultz G, Sharp JG: The effect of the route of delivery of urogastrone on intestinal regeneration. Surgery 1989, 106: 45–51
15. Morin NJ, Laurent G, Nonclercq D, Toubeau G, Heuson Stiennon JA, Bergeron MG, Beauchamp D: Epidermal growth factor accelerates renal tissue repair in a model of gentamicin nephrotoxicity in rats. Am J Physiol 1992, 263:F806–F811
16. Juhl CO, Vinter-Jensen L, Jensen LS, Dajani EZ: Preventive effects of recombinant human epidermal

growth factor on the oesophageal epithelium in pigs subjected to sclerotherapy. Eur J Hepatol Gastroenterol 1995 (in press)
17. Jensen LS, Krarup N, Larsen JA, Juhl C, Nielsen TH: Effect of acute portal hypertension on hepatosplanchnic hemodynamics and liver function. Scand J Gastroenterol 1986, 22:463–470
18. Baserga R: Growth regulation of the PCNA gene. J Cell Sci 1991, 98:433–436
19. Nexø E, Jørgensen E, Hansen MR: Human epidermal growth factor on molecular forms present in urine and blood. Regul Pept 1992, 42:75–84
20. Jørgensen PE, Rasmussen TN, Skov Olsen P, Raaberg L, Seier Poulsen S, Nexø E: Renal uptake and excretion of epidermal growth factor from plasma in the rat. Regul Pept 1990, 28:273–281
21. Vaughan TJ, Pascall JC, James PS, Brown KD: Expression of epidermal growth factor and its mRNA in pig kidney, pancreas and other tissues. Biochem J 1991, 279:315–318
22. Strayer DS, Shijun Y, Schwartz MS: Epidermal growth factor-like growth factors. I. Breast malignancies and other epithelial proliferations in transgenic mice. Lab Invest 1993, 69:660–673
23. Møller JC, Djurhuus JC: Obstructive disease of the kidney (hydronephrosis) and the urinary tract. Renal Pathology: With Clinical and Functional Correlations. Edited by C Tisher, BM Brenner. Philadelphia, J.B. Lippincott Company, 1994, pp 863–894
24. Riden DJ, Kirby JD, Maizels M: Expression of transforming growth factor β and epidermal growth receptor mRNA in UPJ obstruction. Proc Am Acad Ped 1993, 62 (abstr.)
25. Olsen PS, Poulsen SS, Kirkegaard P, Nexø E: Role of submandibular saliva and epidermal growth factor in gastric cytoprotection. Gastroenterology 1984, 87: 103–108
26. Vinter-Jensen L, Frøkiær J, Jørgensen PE, Marqversen J, Rehling M, Dajani EZ, Nexø E: Tissue distribution of ^{131}I-epidermal growth factor in the pig visualized by dynamic scintigraphy. J Endocrinol 1995, 144:5–12
27. Guglietta A, Lesch CA, Romano M, McClure RW, Coffey RJ: Effect of transforming growth factor-α on gastric acid secretion in rats and monkeys. Dig Dis Sci 1994, 39:177–182.
28. Southgate J, Hutton KAR, Thomas EFM, Trejdesiewicz L: Normal human urothelial cells *in vitro*: proliferation and induction of stratification. Lab Invest 1994, 71:583–594
29. Hall PA, Coates PJ, Goodlad RA, Hart IR, Lane DP: Proliferating cell nuclear antigen expression in non-cycling cells may be induced by growth factors *in vivo*. Br J Cancer 1994, 70:244–247
30. Parsons G, Mulholland SG: Bladder surface mucin. Am J Pathol 1978, 93:423–432
31. Gill WB, Ruggioro K, Strauss H: Crystallization studies in a urothelial-lined living test tube (the catheterized female rat bladder). Invest Urol 1979, 17:257–261
32. Remy L: The intracellular lumen: origin, role and implications of a cytoplasmic neostructure. Int Biol Cell 1986, 56:97–106
33. Quincey C, Raitt N, Bell J, Ellis IO: Intracytoplasmic lumina: a useful diagnostic feature of adenocarcinomas. Histopathology 1991, 19:83–87
34. Sobrinho-Simoes M: The diagnostic significance of intracytoplasmic lumina in metastatic neoplasms. Ultrastruct Pathol 1981, 2:327–335
35. Alroy J, Pauli BU, Hayden JE, Gould VE: Intracytoplasmic lumina in bladder carcinomas. Hum Pathol 1979, 10:549–555