Korean J. of Pharmacology
Vol. 29, No. 1, pp. 73-83, 1993

Effect of β-Estradiol on the Growth of Primary Rabbit Proximal Tubule Cells in Serum-Free Medium

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ABSTRACT

In order to examine the effect of β-estradiol on the cell growth, using a primary rabbit kidney proximal tubule cell culture system. We investigated the effect of β-estradiol on alpha 1(IV) collagen and β-actin mRNA levels from primary rabbit kidney cell cultures, and also the effects of 3 growth factors and β-estradiol supplementation on the growth of primary rabbit kidney proximal tubule cells in the serum-free medium. 1 nM of β-estradiol showed a sizable potentiation effect on the growth of the proximal tubule cell in serum-free medium, but higher concentration (>10 nM) of estradiol indeed inhibited the growth. In the absence of hydrocortisone as a growth supplement in serum-free medium, β-estradiol caused to potentiate the growth of the cell. In the presence of hydrocortisone, β-estradiol also potentiated the growth of the proximal tubule cells. According to the Northern analysis, β-estradiol increased the level of β-actin mRNA, although mRNA level of the alpha I(IV) collagen was not changed significantly.

Key Words: β-Estradiol, Cell growth, Primary rabbit kidney proximal tubule cell, Serum-free medium, Growth factor, β-Actin mRNA

INTRODUCTION

Growth supplements have been identified that permit a number of established animal cell lines to grow in the serum-free medium. In many cases the cells can grow in the serum-free medium at the same rate as that obtained with serum supplemented medium (Hayashi and Sato, 1976; Hutchings and Sato, 1978; Mather and Sato, 1979; Taub *et al.*, 1979). Long-term growth with periodic subculturing can be obtained a number of established animal cell lines when using the appropriate hormone-supplemented serum-free medium (Ambesi-Impiombato *et al.*, 1980; Hayashi *et al.*, 1978; Hutchings and Sato, 1978; Mather, 1980; Taub *et al.*, 1979). In a number of cases clonal growth of animal cells can be obtained in the serum-free medium (Bettger *et al.*, 1981; Kaighn *et al.*, 1989; Rizzino, 1987; Taub and Sato, 1980).

Of particular impotance is the fact that the hormonally defined media developed for a number of established animal cell lines often permit the maintenance and/or growth of primary cultures of differentiated cells derived from the same tissue (Bottenstein *et al.*, 1979; Darmon *et al.*, 1981; Mather, 1984; Orly *et al.*, 1980; Taub *et al.*, 1979).

Growth factors have been used for a number of established cell lines include EGF (Hutchings and Sato, 1978), nerve growth factor (Mather and Sato,

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1979) and FGF (Wolfe *et al.*, 1980). Classical hormones are often also critical supplements for serum-free growth, including steroids such as hydrocortisone and peptide hormones such as parathyroid hormone. In a number of cases the groups of supplements that are required for the growth of a particular established cell line also permit the growth of primary cultures of differentiated cells that originate from the same tissue maintained in the serum-free medium.

A primary rabbit kidney epithelial cell-culture system has been developed in serum-free medium. In this system, the epithelial cell express the functions such as proximal tubule functions (Chung *et al.*, 1982). Growth-stimulatory factors for primary rabbit kidney proximal tubule cells include insulin, transferrin, hydrocortisone and FGF (Wolfe *et al.*, 1980). A hormonally defined medium for the LLC-PK1 cell line, which also exhibits proximal-tubule transport functions, was also developed, but some growth requirements differ from those of primary rabbit kidney cells (Chunman *et al.*, 1982). This difference may possibly be related to the observation that LLC-PK1 cells possess some hormone responses typical of distal tubule cells rather than proximal tubule cells.

Hormonally defined media have been developed for the growth of several tumorigenic human epithelial cell lines, including HeLa (Hutchings and Sato, 1978), MCF-7 (Barnes and Sato, 1979), ZR-75-1 (Allegra and Lippman, 1978) and T84 (Murakami and Masui, 1980).

It has been reported that primary rabbit kidney cells grow without fibroblast overgrowth in serum-free medium supplemented with insulin, tranferrin and hydrocortisone (Chung *et al.*, 1982; Jung *et al.*, 1992). Confluent monolayers of this culture cell could retain such proximal tubule transport functions as a sodium/glucose cotransport (Chung *et al.*, 1982; Sakhrani *et al.*, 1984), a sodium dependent phosphate transport (Waqar *et al.*, 1988), and a *p*-aminohippurate transport (Yang *et al.*, 1988).

As mentioned above, although there has been reported that insulin, transferrin, hydrocortisone, triiodothyronine, prostaglandin, EGF and FGF have a role as growth factors in the serum-free media for the growth of several cells such as kidney proximal tubular epithelial cells and tumorigenic human epithelial cell lines, there is yet no report to show the effect of β-estradiol steroid hormone on the growth of the renal tubular epithelial cells.

In order to examine the effect of β-estradiol on the cell growth, using a primary rabbit kidney proximal tubule cell culture system. We investigated the effect of β-estradiol on alpha I (IV) collagen and β-actin mRNA levels from primary kidney cell cultures, and also the effects of 3 growth factors and β-estradiol supplememtation on the growth of primary rabbit kidney proximal tubule cells in the serum-free medium.

MATERIALS AND METHODS

Materials

Hormones, human transferrin, trypsin EDTA (10×) and other chemicals were purchased from Sigma Chemical Corp. (St. Loius, Mo.), powdered medium and soybean trypsin inhibitor were from Life Technologies (Grand Island, NY), class IV collagenase was from Worthington (Freehold, NJ), gamma ^{32}P-ATP, alpha ^{32}P-dCTP (3,000 Ci/mmol) and random priming labelling kits were purchased from Dupont/NEN. Restriction endonucleases were obtained from Promega, 1 kb DNA ladder from Life Technologies, Inc., and Zeta Probe Blotting Membrane from Biorad. Liquiscint was obtained from National Diagnostics (Parsippany, NY). Iron oxide was prepared by the method of Cook and Pickering (1958). Iron oxide stock solutions in 0.9% NaCl were sterilized using an autoclave, and diluted with phosphate buffered saline (PBS, pH 7.4) prior to use.

Cell culture environment

Primary rabbit kidney proximal tubule cells were maintained in a 5% CO_2 humidified environment at 37℃ by a serum-free basal medium supplemented with β-estradiol and 3 growth supplements, 5 μg/ml insulin, 5 μg/ml transferrin and 5×10^{-8} M hydrocoritsone (Chung *et al.*, 1982; Jung *et al.*, 1992) or 2 growth supplements (minus hydrocortisone). The basal medium (pH 7.4) used for primary rabbit kidney proximal tubule cell culture (DME/F12) consisted of a 50:50 mixture of Dulbecco's Modified Eagle's Medium and Ham's F12 Medium containing 15 mM HEPES buffer,

and 20 mM sodium bicarbonate. Immediately prior to the use of medium, the 2 or 3 growth supplements and β-estradiol were added. Water utilized in medium preparation was purified by mean of a Millique Deionization System.

Primary rabbit kidney proximal tubule cell culture

Primary rabbit kidney proximal tubule cells were prepared by a modification of the method of Chung *et al.* (1982). To summarize, the kidneys of a male New Zealand white rabbit (2 to 2.5 kg) were perfused via the renal artery, first with Phosphate Buffered Saline (PBS), and subsequently with DME/F12 containing 0.5% iron oxide (wt/vol) till the kidney truned grey-black in color. Renal cortical slices were homogenized with 4 strokes of a sterile Dounce homogenizer (type A pestle), and the homogenate was poured first through a 253 μ and then a 83 μ mesh filter. Tubules and glomeruli on top of the 83 μ filter were transferred into sterile DME/F12 medium containing a magnetic stir bar. Glomeruli (containing iron oxide) were removed with the stir bar. The remaining purified proximal tubules were briefly incubated in DME/F12 containing 0.124 mg/ml collagenase (class IV), and 2.5 mg% soybean trypsin inhibitor. The tubules were then washed with DME/F12 medium by centrifugation and resuspended in DME/F12 containing the 3 supplements, and transferred into tissue culture dishes. Medium was changed one day after plating and every two days thereafter.

Cell growth studies

Primary proximal tubule cell cultures were cultured in 35 mm dishes for cell growth studies. Periodically, cells were removed from the dishes using Phosphate Buffered Saline containing 0.05% trypsin, and 0.5 mM EDTA. The cells were counted using a Coulter Model ZF particle counter. Values are expressed as the average of triplicate determinations.

Northern analysis of cellular RNA

Total RNA was isolated by the guanidinium isothiocyanate/cesium chloride method (Chirgwin *et al.*, 1979). RNA was isolated from primary rabbit kidney proximal tubule cells grown to confluency either in DME/F12 supplemented with insulin, transferrin, hydrocortisone and β-estradiol, or in DME/F12 supplemented with β-estradiol, in addition to the other 2 growth factors (minus hydrocortisone). RNA (10 μg/sample) was fractionated by electrophoresis in formaldehyde gels containing 0.8% agarose, and was tranferred to Zeta Probe Blotting Membranes. Duplicate RNA samples on the gel were stained with ethidium bromide to verify the quality of the RNA. A restriction fragment containing mouse alpha I (IV) collagen cDNA obtained from plasmid pE123 (Boot-Handford *et al.*, 1987), and a restriction fragment containing mouse β-actin cDNA obtained from plasmid pBR322 (Boot-Handford *et al.*, 1987) were utilized for making labelled probes. Plasmids pE123 and pBR322 were kindly provided by Dr. Marceau Kirkinin. The

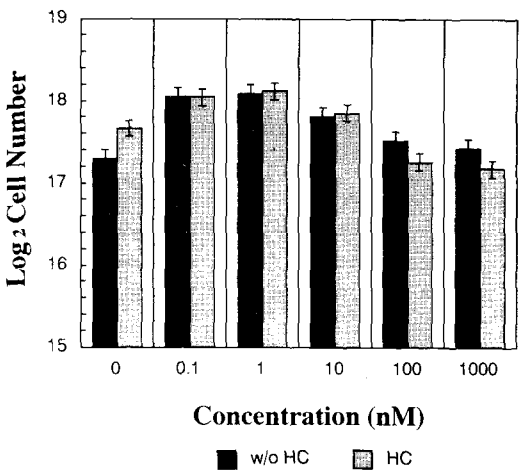

Fig. 1. Effect of β-Estradiol on Growth of Primary Rabbit Kidney Proximal Tubule Cells by Different Concentration of β-Estradiol. The cells were cultured in DME/F12 supplememted either with 5 μg/ml insulin, 5 μg/ml transferrin and 0~1000 nM β-estradiol (■), or with an additional 5×10^{-8} M hydrocortisone (▦). The cell number present in each growth conditions was determined after 4 days in culture using a Coulter Counter. Values are the average (± Std. deviation) of triplicate determinations: w/o HC: without hydrocortisone in culture medium. HC: With hydrocortisone in culture medium.

restriction fragments were radiolabelled with alpha ^{32}P-dCTP by the random primer method, and were utilized for hybridization following the method of Church and Gilbert (1984). Standard stringent hybridization conditions were utilized.

RESULTS

The effect of β-estradiol on the growth of the rabbit kidney primary proximal tubule cells in the serum-free medium

Figures 1, 2 and 3 showed the effects of different concentrations of β-estradiol on the growth of primary rabbit kidney proximal tubule cells, whose growth was determined after 4, 8 and 12 days, respectively. In the 0.1 or 1 nM β-estradiol containing medium, cell growth was augmented, but cell growth was inhibited with more than 10 nM concentration of β-estradiol. Therefore 1 nM of β-estradiol was thought to be a proper concentration for the growth of the proximal tubule cell. When we determined the effects of β-estradiol on the cell growth in the absence or presence of the hydrocortisone, no significant difference between two groups were observed.

The effect of hydrocortisone on growth of primary rabbit kidney proximal tubule cells

As shown in figure 4 hydrocortisone, which is one of the growth factors, augmented the cell growth after 8 and 12 days in culture, In the mean time after 4 days in culture, hydrocortisone did not potentiate the cell growth significantly.

The effect of β-estradiol on growth of primary rabbit kidney proximal tubule cells in without hydrocortisone

Figure 5 showed the effects of β-estradiol on the growth of the rabbit kidney primary proximal tubule cell in the serum-free medium without hydrocortisone. As shown in the figure 5, 1 nM β-

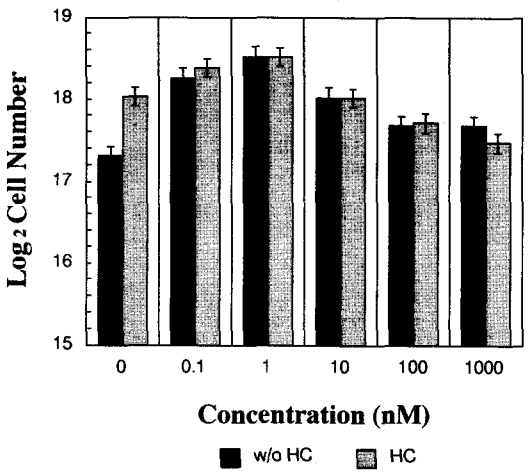

Fig. 2. Effect of β-Estradiol on Growth of Primary Rabbit Kidney Proximal Tubule Cells by Different Concentration of β-Estradiol. The cells were cultured in DME/F12 supplememeted either with 5 μg/ml insulin, 5 μg/ml transferrin and 0~1000 nM β-estradiol (■), or with an additional 5×10^{-8} M hydrocortisone (▩). The cell number present in each growth conditions was determined after 8 days in culture using a Coulter Counter. Values are the average (± Std. deviation) of triplicate determinations.

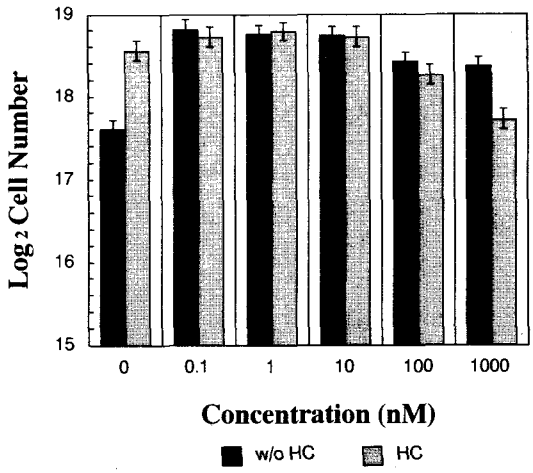

Fig. 3. Effect of β-Estradiol on Growth of Primary Rabbit Kidney Proximal Tubule Cells by Different Concentration of β-Estradiol. The cells were cultured in DME/F12 supplememted either with 5 μg/ml insulin, 5 μg/ml transferrin and 0~1000 nM β-estradiol (■), or with an additional 5×10^{-8} M hydrocortisone (▩). The cell number present in each growth conditions was determined after 12 days in culture using a Coulter Counter. Values are the average (± Std. deviation) of triplicate determinations.

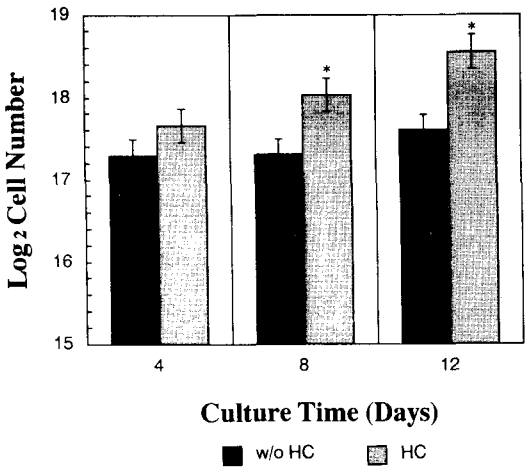

Fig. 4. Effect of Hydrocortisone on Growth of Primary Rabbit Kidney Proximal Tubule Cells. The cells were cultured in DME/F12 supplememted either with 5 μg/ml insulin and 5 μg/ml transferrin (■), or with an additional 5×10^{-8} M hydrocortisone (▦). The cell number present in each growth conditions was determined after 4, 8 and 12 days in culture using a Coulter Counter. Values are the average (± Sdt. deviation) of triplicate determinations. *Significantly different from the group of without hydrocortisone, respectively ($P<0.05$).

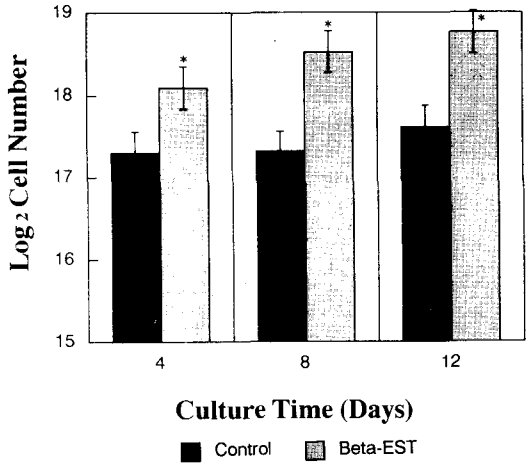

Fig. 5. Effect of β-Estradiol on Growth of Primary Rabbit Kidney Proximal Tubule Cells in Without Hydrocortisone Medium. Primary proximal tubule cells were grown in 35 mm dishes containing either β-estradiol free DME/F12 or DME/F12 supplemented with 1.0 nM β-estradiol. Both the β-estradiol free and β-estradiol containing culture medium was further supplemented with 2 growth factors, insulin and transferrin. Cells were counted periodically over a 12 day time interval. Values are the average (±Std. deviation) of triplicate determinations. *Significantly different from the group of without β-estradiol, respectively ($P<0.05$).

estradiol significantly potentiated the cell growth after 4, 8 and 12 days in culture suplemented with two growth factors, insulin and transferrin when compared with the growth without β-estradiol.

The effect of β-estradiol on growth of primary rabbit kidney proximal tubule cells in additional hydrocortisone medium

Figure 6 showed the same result as figure 5 except that in figure 6 we represented the effect of β-estradiol on the cell growth in the presence of hydrocortisone. As illustrated in figure 6, 1 nM of β-estradiol was also cell growth stimulator in serum-free DME/F12 medium supplemented with insulin, transferrin and hydrocortisone.

We compared the difference of effect of β-estradiol on the cell growth in serum-free medium supplemented either with two growth factors (insulin and transferrin) or with three growth factors (two factors and hydrocortisone).

Although we already observed the stimulatory effect of β-estradiol on the cell growth in medium with or without hydrocortisone, there was no significant difference in degree of stimulatory actions between hydrocortisone free and containing medium. Therefore β-estradiol was thought to be a growth stimulatory factor whether hydrocortisone is present in the culture medium or not (Figure 7).

The effect of β-estradiol on alpha I (IV) collagen and β-actin mRNA contents

Alpha I (IV) collagen and β-actin mRNAs were transferred to Zetabind blotting membrane and hybridized sequentially to β-actin or alpha I (IV) collagen specific radiolabeled probes. EtBR-

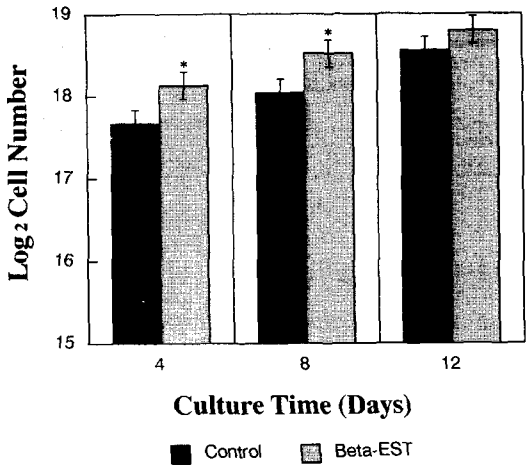

Fig. 6. Effect of β-Estradiol on Growth of Primary Rabbit Kidney Proximal Tubule Cells in Additional Hydrocortisone Medium. Primary proximal tubule cells were grown in 35 mm dishes containing either β-estradiol free DME/F12 or DME/F12 supplemented with 1.0 nM β-estradiol. Both the β-estradiol free and β-estradiol containing culture medium was further supplemented with 3 growth factors, insulin, transferrin and hydrocortisone. Cells were counted periodically over a 12 day time interval. Values are the average (±Std. deviation) of triplicate determinations. *Significantly different from the group of without β-estradiol, respectively ($P<0.05$).

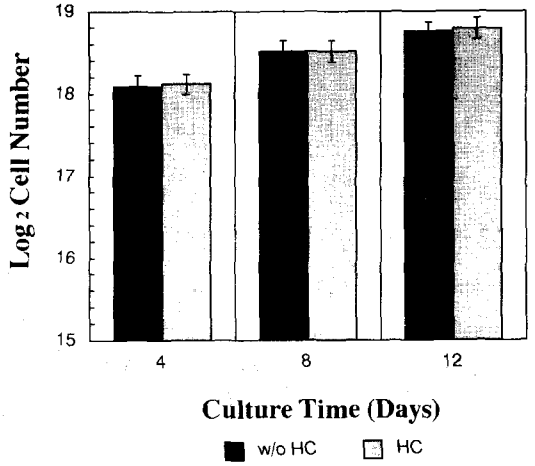

Fig. 7. Effect of β-Estradiol on Growth. Primary proximal tubule cells were grown in 35 mm dishes containing either hydrocortisone free DME/F12 or DME/F12 supplemented with 5×10^{-8} M hydrocortisone. Both the hydrocortisone free and hydrocortisone containing culture medium was further supplemented with 2 growth factors, insulin and transferrin. Cells were counted periodically over a 12 day time interval. Values are the average (±Std. deviation) of triplicate determinations.

stained RNA samples (Fig. 8, lower panel) established minimal variation in sample recovery; as judged by the bands representing the major ribosomal RNA species (28 S and 18 S), there was negligible breakdown.

The effect of exogenous β-estradiol on the levels of alpha I (IV) collagen and β-actin chain mRNAs in primary rabbit kidney proximal tubule cells was examined by Northern analysis using mouse cDNA and nick-translated cDNA probes. Fig. 8, top panel shows that the level of β-actin mRNA was elevated in primary cultures in medium containing 1 nM β-estradiol (Gel 2; B, D), as compared with β-estradiol free medium (Gel 1; A, C). In contrast alpha I (IV) collagen chain mRNA levels did not differ significantly either in β-estradiol free, or in β-estradiol containing medium.

DISCUSSION

Like androgen, β-estradiol and estrogen have nitrogen retention properties, but sodium and water retention properties of β-estradiol and estrogen are weaker than those of androgen. The analbolic actions of estrogens are mediated by the same receptor protein that mediates the actions of the hormone in other target tissues (Saartok *et al.*, 1984).

Estrogens, like other steroids, act by regulating the transcription of a limited number of genes. The hormones diffuse passively through cell membranes, distribute themselves throughout the cell, and ultimately bind to the nuclear estrogen receptor (Beato, 1989; Evans, 1988; Gorsk *et al.*, 1986). The presence of the estrogen receptor in a

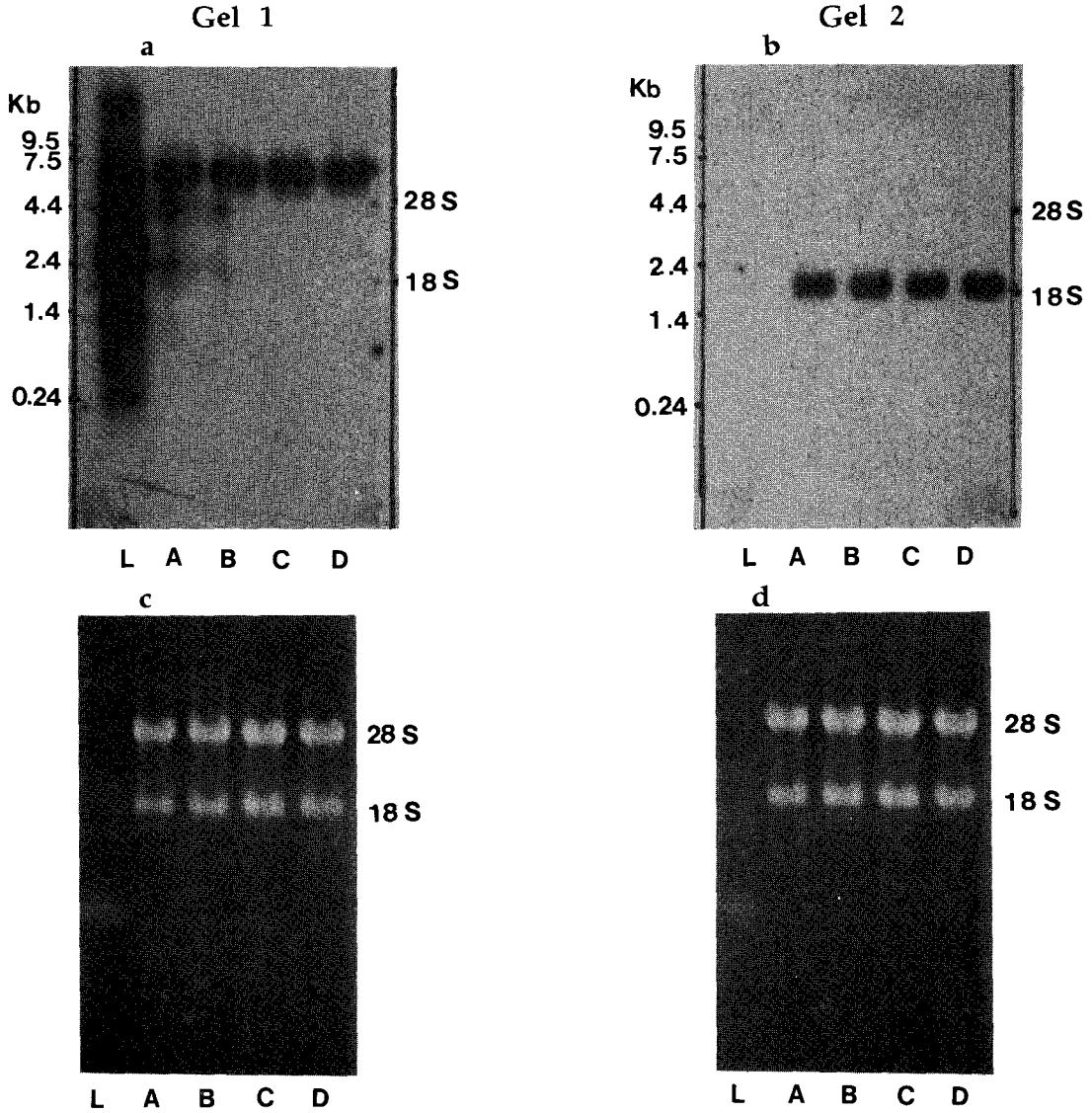

Fig. 8. Northern Blot Analysis of Alpha I (IV) Collagen and β-Actin mRNA from Primary Kidney Cell Cultures. RNA was isolated from primary cultures grown to confluency in DME/F12 with the 2 or 3 supplements, and β-Estradiol at either 1.0 nM. Total RNA (5 μg/lane) was subjected to electrophoresis in 0.8% agarose gel. Duplicate sets of samples were run on each of two gels, gel I and gel II. One set of samples both from gel I and from gel II was transferred to Zetabind blotting membrane. Blots were hybridized with either an alpha I (IV) collagen cDNA probe in the case of gel I, or a β-actin chain cDNA probe in the case of gel II. The blots were washed, and exposed to X-ray film for 24 hours. The results with the alpha I (IV) collagen probe (gel I) and the β-actin probe (gel II) are illustrated in a and b, respectively. The other set of RNA samples both on gel I and gel II was stained with ethidium bromide, as illustrated in c and d, respectively. L: RNA ladder, A: Without hydrocortisone control, B: Without hydrocortisone, add β-estradiol (1.0 nM), C: With hydrocortisone control, D: With hydrocortisone and β-Estradiol (1.0 nM).

tissue is presumptive of the biological actions of estrogen at that location. The presence of estradiol increases via protein synthesis pathways the concentration of receptors for both progesterone and estradiol, while progesterone generally leads to a down regulation of receptor binding sites for these steroids)Evans and Leavitt, 1980).

Since 1936 (Lacassagen), estrogen has been shown to cause multiple tumors in various organs such as breast, uterus, testicle, kidney and bone, and to increase the frequency of tumor incidence. And there is also a definite relation between estrogen and benign hepatoma (Baum *et al.*, 1973; Bergkvist *et al.*, 1989; Greenberg *et al.*, 1981; Kay, 1977; Leis *et al.*, 1976; Lawson *et al.*, 1981; Silverberh and Makowski, 1975; Weiss and Sayvetz, 1980).

Several different hormonally defined media are available for kidney epithelial cell culture. The cell line can grow at the same rate in serum-free medium supplemented with insulin, transferrin, triiodothyronine, hydrocortisone and prostaglandin E_1 as the rate obtained in serum supplemented medium (Taub *et al.*, 1979). Both the long-term growth of MDCK cells and clonal growth can be obtained in serum-free medium supplemented with insulin, transferrin, T_3, hydrocortisone and prostaglandin E_1.

The hormone-supplemented serum-free medium for MDCK cells has also been used for primary cultures of baby-mouse kidney epithelial cells without fibroblast overgrowth (Taub *et al.*, 1979; Taub and Sato, 1980). The primary baby-mouse kidney epithelial cells exhibit functional properties like MDCK cells, but lack sodium-dependent sugar transport which is an unique function of a proximal tubule function.

A particular strain of HeLa grows in serum-free medium supplemented with insulin, transferrin, EGF, FGF and hydrocortisone with serial subculturing, at the same rate as that obtained with serum supplemented medium (Hutchings and Sato, 1978). Hydrocortisone is the most critical factor for the long-term serum-free growth of these HeLa cells (Hayashi *et al.*, 1978). Hormonally defined media have been developed for two different established human mammary-tumor cell line, ZR-75-1 and MCF-7. Whereas ZR-75-1 cells require insulin, transferrin, FGF, hydrocortisone and triiodothyronine for serum-free growth (Barnes and Sato, 1979), MCF-7 cells require insulin, transferrin, EGF and prostaglandin $F_{2\alpha}$, as well as several attachment factors (Allegra and Lippman, 1978).

Frowth-stimulatory factors for primary culture of rabbit kidney proximal tubule cells such as insulin, transferrin and hydrocortisone has been well studied. A hormonally defined medium for the MDCK and LLC-PK1 cell-line, which also exhibits proximal-tubule transport functions, was also developed, but the growth requirements differ from those of primary rabbit kidney cells (Chunman *et al.*, 1982). This difference may possibly be related to the observation that LLC-PK1 cells possess some hormone responses typical of distal tubule cells, rather than proximal tubule cells.

Although many investigators already showed the effect of steroid hormones as growth stimulator on cell growth, there is no report to reveal the capacity of β-estradiol in serum free medium as a cell growth factor in rabbit kidney primary proximal tubule cell culture.

There are several reports to show the possible role of steroid hormones as growth supplements in defined growth media: e.g β-estradiol in human breast cancer cell line (Allegra and Lippman, 1978), hydrocortisone in HeLa cell (Hutchings and Sato, 1978), progesterone in rat neuroblastoma cell line (Bottenstein and Sato, 1979), and testosterone in mouse prostatic epithelial cell (Waymouth *et al.*, 1982).

Connective tissues contain a number of species of collagens, glycoproteins, and proteoglycans (Bornstein and Sage, 1980; Kleinman *et al.*, 1986). These macromolecules form the matrix structures that contribute to the physical characteristics of tissues as well as provide unique substrates for the resident cells. Basement membranes contain type IV collagen (Kefalides, 1973; Orkin *et al.*, 1976), the glycoproteins laminin (Timpl *et al.*, 1979), entactin (Carlin *et al.*, 1981), and nidogen (Timpl *et al.*, 1983) and heparan sulfate proteoglycan (Kanwar & Farquhar, 1979; Hassell *et al.*, 1980, 1985). β-actin is a major structural protein of cellular microfilaments, it is considered to be involved in cell motility and mitosis.

Cell attachment factors are often required for the attachment and growth of animal cells in serum-free medium. Cell attachment factors that

were subsequently used successfully include the extracellular matrix proteins laminin and collagen.

The major high-molecular-weight extracellular matrix proteins present in Matrigel have been characterized, and include laminin, collagen IV, heparan sulphate proteoglycan, entactin and nidogen (Kleinman *et al.*, 1985).

In this study we investigated the effect of β-estradiol on cell growth in serum-free medium supplememted with insulin, transferrin and hydrocortisone. β-estradiol indeed increased the proliferation of rabbit proximal tubule cell whether hydrocortisone is present in the growth medium or not, and according to the Northern analysis mRNA level of β-actin was increased though there was no measurable change in alpha I (IV) collagen mRNA level.

Therefore we may conclude that growth stimulatory property of β-estradiol ascribed to the stimulation of the protein synthesis pathway, which is a primary component of cellular microfilament.

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=국문초록=

β-Estradiol이 토끼 근위 세뇨관 상피세포의 성장에 미치는 영향

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Steroid hormone의 하나인 β-estradiol이 serum-free medium에서 배양한 토끼의 신장 근위세뇨관 상피세포의 세포성장과 기능에 미치는 영향을 관찰한 바 다음과 같은 결과를 얻었다.

1. Serum-free medium에서 토끼의 신장 근위세뇨관 상피세포는 β-estradiol 1 nM의 농도에서 유의한 세포 성장 촉진 효과를 나타내었고, β-estradiol 10 nM이상의 농도에서는 세포성장이 억제되었다.

2. β-Estradiol은 serum-free medium에서 성장촉진인자의 하나인 hydrocortisone을 뺀 조건하에서 세포 성장을 증가시키었다.

3. β-Estradiol은 hydrocortisone을 growth supplement로 넣어준 serum-free medium에서 토끼 신장의 근위세뇨관 상피세포의 성장을 촉진시키었다.

4. β-Estradiol은 Northern blot analysis에 의하여 확인한 alpha I (IV) collagen mRNA level에는 별다른 변화를 보이지 않으나, β-actin mRNA level은 증가되었다.

이상의 결과로 미루어 보아, serum-free 그리고 hormonally defined media에서 β-estradiol이 토끼의 신장 근위세뇨관 상피세포의 성장 및 기능에 대하여 촉진적으로 작용하는 것은 cellular microfilament의 중요한 구성단백의 하나로 밝혀진 β-actin의 합성 증가에 기인하는 것으로 생각된다.