Journal of Steroid Biochemistry Vol. 10, pp. 123 to 128

0022-4731/79/0201-0123$02.00/0

ESTROGEN RECEPTOR IN HAMSTER KIDNEY DURING ESTROGEN-INDUCED RENAL TUMORIGENESIS

NORMAN S. ANDERSON III*, YVONNE DAVID and DARRELL D. FANESTIL†
Department of Medicine, M-013, University of California, San Diego, La Jolla, CA 92093, U.S.A.

(*Received* 31 *July* 1978)

SUMMARY

Specific binding of [^{3}H]-estradiol to isolated cytosol and to nuclei in tissue slices was determined in hamster kidney before and during estrogen-induced renal tumorigenesis and in the kidney of normal rats, a species which does not develop the tumor. At 10^{-9} M [^{3}H]-estradiol, cytosol from normal hamster kidney bound $6.48 \pm 0.3 \times 10^{-15}$ mol/mg protein, which was significantly less than the $15.8 \pm 1.1 \times 10^{-15}$ mol/mg bound by the rat. The apparent dissociation constant for estradiol was not significantly different (0.50 ± 0.08 nM in hamster and 0.66 ± 0.14 nM in rat). Cytosol binding increased after hamsters were implanted with estrogen pellets for 6 months ($9.23 \pm 0.2 \times 10^{-15}$ mol/mg) or had developed tumors ($13.7 \pm 3.5 \times 10^{-15}$ mol/mg). In tissue slices, the amount of [^{3}H]-estradiol translocated to nuclei was greater in the rat ($21.8 \pm 0.8 \times 10^{-14}$ mol/mg nuclear protein) than in the normal hamster ($3.28 \pm 0.96 \times 10^{-14}$ mol/mg) and was greater in tumor-bearing hamsters ($6.70 \pm 0.86 \times 10^{-15}$ mol/mg DNA) than in normal hamsters ($3.19 \pm 0.63 \times 10^{-15}$ mol/mg DNA). We conclude: (1) the kidney of the tumor prone hamster does not contain an estrogen receptor of excessive quantity or unusual affinity and (2) the estrogen–receptor complex in estrogen-induced tumors can be translocated to cellular nuclei.

INTRODUCTION

The characteristics and morphology of the estrogen-induced and estrogen-dependent renal carcinoma of the male Golden Syrian hamster have been described in considerable detail by Kirkman[1] and by others [2]. In this experimental renal tumor, tumor growth can be induced by a variety of estrogens, while concurrent treatment with progesterone, deoxycorticosterone [1], 2α-bromoergocryptine [3], or the anti-esterogen nafoxidine [4] inhibits tumor formation. Although the induction of the renal tumor with estrogens and responsiveness of the tumor to steroid hormone manipulation suggest a direct action of estradiol in promoting the tumorigenesis, this postulate remains speculative [3]. At present, the best evidence for such a direct action of estrogen in hamster renal tumorigenesis has been from studies which have related estrogen binding in renal cytosol with development of renal tumors [5–8]. However, the rat is another species whose kidneys also contain estrogen-binding macromolecules [9], but it does not develop the estrogen-induced renal tumor.

In early studies of the hamster renal tumor and estrogen binding, Steggles and King[6] were unable to demonstrate significant cytoplasmic or nuclear [^{3}H]-estradiol binding either *in vitro* or *in vivo*. However, recently Li *et al.*[7] have demonstrated [^{3}H]-estradiol binding in normal hamster kidney and reported a small but significant increase in renal cytoplasmic binding of [^{3}H]-estradiol when hamsters were treated with estradiol or diethylstilbesterol for 6–10 months. Additionally, Li *et al.*[7] related the increase in cytoplasmic binding of [^{3}H]-estradiol to the length of E_2 or DES treatment.

Our studies were initiated to investigate further the cytoplasmic binding of estradiol in kidney during the period of tumorigenesis [10] and to relate this with nuclear binding during the same time period. Our purpose was two-fold: first, to determine if the hamster kidney is able to bind an unusual quantity of estrogen, suggesting that this species has a propensity for estrogen-mediated actions on the kidney; and, second, to determine if the receptors for estrogen binding in normal hamster kidney and kidney tumor have the ability to translocate estrogen into the nucleus, a necessary step if the estrogen acts directly on the kidney or tumor via the established mode of action of steroid hormones in normal tissues.

EXPERIMENTAL

Tritium labelled estradiol, [2,4,6,7-^{3}H]-estradiol (91 Ci/mmol), and [2,4,6,7,16-^{3}H(N)]-estradiol (152 Ci/mmol) were obtained from New England Nuclear, Boston, Massachusetts. All nonradioactive steroids and reagents used in the study were obtained from the Sigma Chemical Company, St Louis, Missouri. Pellets of 17β-estradiol (25 mg) were prepared by the UCLA School of Pharmacy, Los Angeles, California.

Animals. Eight-week old male Golden Syrian hamsters (LSH SS/LAK Lakeview Hamster Colony, Newfield, New Jersey) and 200–225 g Sprague–Dawley

* Present address: Department of Anatomy/School of Medicine, University of Oregon Health Sciences Center, Portland, OR 97201, U.S.A.
† To whom correspondence and reprint requests should be addressed.

rats (Charles River Breeding Laboratories, Wilmington, Massachusetts) were housed under controlled conditions and given tap water and lab chow *ad libitum* during the course of the experiments.

The hamsters were divided equally and randomly into two separate groups; the animals in one group were implanted with a pellet containing 25 mg of 17β-estradiol (E_2) and the hamsters in the other group served as age-matched controls. The estrogen pellets were inserted subpannicularly through a small incision at the base of the tail and implanted between the scapulae. Reimplantation of this group with an estrogen pellet was performed after 5–6 months to insure constant treatment with E_2. In all experiments, E_2 pellets were removed from the treated animals at least 68 h prior to sacrifice. The age-control hamsters and Sprague–Dawley rats received no treatment.

All animals were sacrificed under sodium pentobarbital anesthesia and were perfused via the heart with phosphate buffer (NaCl 135 mM, KH_2PO_4 5 mM, Tris base 5 mM, $MgCl_2$ 0.5 mM, $CaCl_2$ 1.0 mM, glucose 5 mM, pH 7.4 at 2°C) after which the kidneys were removed, decapsulated and rinsed in the same buffer at 0°C.

Cytosol binding studies. Kidneys were homogenized with a Teflon–glass homogenizer in a volume of 0.01 Tris, 0.001 M EDTA pH 7.4 (Tris EDTA) buffer equal to 5 times the weight of the tissue in grams (5 vol.) and the resulting homogenate was centrifuged at 105,000 *g* for 1 h at 0–4°C. Fractions of the 105,000 *g* supernatant fraction (cytosol) were then incubated at 0°C in the presence of various concentrations of [^{3}H]-estradiol both in the absence and in the presence of a 1000-fold molar excess of nonradioactive estradiol in order to calculate the [^{3}H]-estradiol which was specifically bound (i.e., displaceable by the nonradioactive steroid)[11]. After incubation of the cytosols with [^{3}H]-estradiol for the appropriate time period, a 100 μl aliquot of 10% charcoal, 1% dextran suspended in Tris–EDTA buffer was added to 500 μl of cytosol previously incubated with [^{3}H]-steroid. The resultant mixture was mixed vigorously for 30 s and allowed to stand at 0°C for 20 min before centrifugation at 0–4°C at 12,000 *g* for 15 min. The supernatant was removed with a Pasteur pipette and radioactivity was quantitated.

Nuclear binding studies. Nuclear accumulation of [^{3}H]-estradiol was studied in a tissue slice system modified from that described by Marver *et al.*[12]. Slices of kidney tissue were prepared with a Sadie–Riggs microtome and were maintained at 0°C in phosphate buffer, pH 7.4. Slices from two kidneys were added to 20 ml of phosphate buffer containing 10^{-9} M [^{3}H]-estradiol with or without 10^{-6} M nonradioactive estradiol. The slices were incubated at 25°C for 1.5 h and the incubation was then terminated by three successive washes in 20 ml of cold (0–4°C) phosphate buffer. The washed slices were then homogenized in a Teflon–glass homogenizer in 0.32 M sucrose 0.003 M $MgCl_2$ and the resulting homogenate centrifuged at 750 *g* at 0–4°C to obtain a crude nuclear pellet. The crude nuclear pellet was resuspended in 5.0 ml of 2.4 M sucrose 0.001 M $MgCl_2$ and centrifuged at 0–4°C in a Beckman SW50.1 rotor at 56,000 *g* for 1 h. Purified nuclei, pelleted with this procedure, were then extracted with 0.1 M Tris and 0.3 M KCl to obtain both "Tris soluble" and chromatin bound [^{3}H]-estradiol [12]. The "Tris soluble" [^{3}H]-estradiol was then partially purified by precipitation of the complex in saturated ammonium sulfate [12].

Protein DNA and radioactivity determination. All proteins were determined by the method of Lowry *et al.*[13]. The DNA content of the nuclear pellet was assessed by the method of Burton[14] with calf thymus DNA as the standard. Aliquots of cytosol or nuclear extracts containing radioactivity from each experiment were counted in a mixture of POP, POPOP and Triton-X and the counts were corrected for quenching with the use of an external standard ratio. The data are presented as moles of [^{3}H]-estradiol specifically bound per mg of protein or DNA.

RESULTS

Cytosol binding of [^{3}H]-estradiol

The time course of the specific binding of [^{3}H]-estradiol in cytosol from normal hamster kidney at 0°C, as shown in Fig. 1, is characterized by an initial rapid increase in specific [^{3}H]-E_2 binding in the first 15–30 min of incubation, followed by a gradual leveling of binding to equilibrium after 2–4 h of incubation.

Accordingly, cytosols derived from the kidneys of normal hamsters (NH), hamsters with renal tumors (TH) and normal rats (NR) were examined for the presence of bound [^{3}H]-estradiol after 2.5 h of incubation with 10^{-9} M [^{3}H]-E_2 ± 10^{-6} M nonradioactive E_2. The binding data obtained from individual animals in these experimental groups are shown in Fig. 2. The specific binding of [^{3}H]-E_2 in NH cytosol,

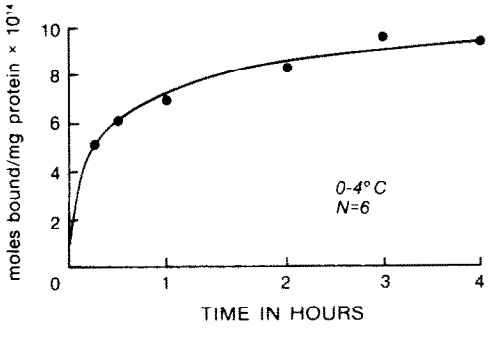

Fig. 1. Time course of the specific binding at 0°C of [^{3}H]-E_2 in cytosol from kidneys of normal hamsters. After homogenization and centrifugation, cytosol was incubated from 15 min to 4 h with 1.0×10^{-9} M [^{3}H]-E_2 ± 10^{-6} M of nonradioactive E_2 in 0.01 M Tris, 0.015 M EDTA, pH 7.4. Unbound steroid was removed with dextran-coated charcoal. Each point represents the mean of four experiments.

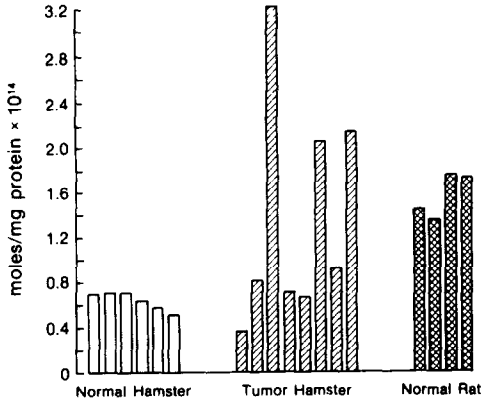

Fig. 2. Cytosol binding of [^{3}H]-estradiol in normal hamster kidney, estrogen-induced renal tumor and normal rat kidney. Each bar represents specific binding in an individual animal analyzed after incubation of cytosol with 1.0×10^{-9} M [^{3}H]-E_2 ± 10^{-7} M nonradioactive E_2.

although low, was relatively constant from animal to animal. The specific binding of [^{3}H]-E_2 in cytosols prepared from NR was also relatively constant but was significantly higher than in kidneys from normal hamsters. In cytosols prepared from the tumor animals, the [^{3}H]-E_2 ranged from 3.82 to 32.26×10^{-15} mol/mg protein. Despite the high degree of variability of the tumor group, the mean group difference in renal cytosol binding of [^{3}H]-E_2 between NH and TH animals was statistically significant (Table 1). Table 1 also contains the kidney cytosol binding of E_2 determined in a fourth group of hamsters, which had been treated with estrogen pellets for 6 months but which did not have identifiable tumor nodules. The binding in the kidney cytosol from these animals was significantly greater than in NH, significantly less than in NR and not significantly different from that in TH. In contrast to the variability in the amount of binding found in cytosol from TH, the cytosol from the 6-month treated hamsters had a small variance.

Possible causes for the variability in [^{3}H]-E_2 binding by renal cytosols prepared from tumor animals was investigated by analyzing the binding of [^{3}H]-E_2 over a 100-fold concentration range (3×10^{-10} M, 6×10^{-10} M ... 3×10^{-8} M) by the method of Scatchard (Fig. 3). The binding of [^{3}H]-E_2 in the normal hamster and normal rat were characterized by having at least two components of [^{3}H]-E_2 binding. The measured K_D of the high affinity component averaged 5.05×10^{-10} M in the normal hamster (Table 2) and 6.65×10^{-10} M in the rat (Table 2). The K_D of the second, low affinity component was difficult to characterize accurately but in each group was of the same order of magnitude.

Binding of [^{3}H]-E_2 in renal cytosols prepared from tumor bearing hamsters was also characterized by the method of Scatchard. As pictured in Fig. 3B, the Scatchard analysis revealed only a single set of sites to which [^{3}H]-estradiol binds after the development of a renal tumor having an average K_D of 7.44×10^{-10} M (Table 2). The significance of this apparent shift from two distinct sites in normal and 6-month treated hamsters to a single affinity site in the tumour-bearing hamsters remains unclear. Although it is possible that a different type of receptor molecule binds [^{3}H]-E_2 in the renal tumor, the affinity of this site for [^{3}H]-estradiol in cytosols prepared from tumor animals was not significantly different from the affinity of the higher affinity site in the normal hamster (Table 3).

Competition studies

The specificity of the binding for steroids with estrogenic activity was studied in a series of experiments. [^{3}H]-E_2 (10^{-9} M) together with 10^{-7} M of several nonradioactive steroids was incubated with cytosols prepared from normal or tumor bearing hamster kidneys. The data pictured in Fig. 4 illustrates the relative ability of these steroids to decrease the amount of bound [^{3}H]-E_2. 17β-Estradiol, estrone and estriol were each effective in decreasing the amount of [^{3}H]-E_2 bound in cytosols from normal or tumor hamsters, while the other steroids tested (testosterone, progesterone and deoxycorticosterone) were without demonstrable effect. Of further interest in Fig. 4 is the finding that the steroid specificity of the proteins which bind [^{3}H]-E_2 in normal or tumor renal cytosols is similar. Despite the similar specificity for estrogenic vs. non-estrogenic compounds, the quantitative extent to which estradiol and estrone decrease the [^{3}H]-E_2 bound is dissimilar in each group of animals. In cytosols prepared from the kidneys of normal hamsters, 10^{-7} estradiol, estrone and estriol each decreased [^{3}H]-estradiol binding by approx. 40%. After treatment of the hamsters with E_2 and development of renal tumors, estradiol, estriol and estrone decreased [^{3}H]-E_2 binding by over 70%. This finding is also supported by the Scatchard analysis of [^{3}H]-E_2 binding in tumor and normal cytosols for the absolute number of sites available for [^{3}H]-E_2 binding would be expected to much larger in NH cytosols due to the presence of the large capacity, low affinity component.

Table 1. Specific binding of [^{3}H]-estradiol in renal cytosols

Source of cytosol	N	10^{-15} mol estradiol bound per mg protein
Normal hamster	6	6.48 ± 0.3*
Hamster treated		
6 months with estrogen	4	9.23 ± 0.2
Tumor hamster	8	13.70 ± 3.5
Normal rat	4	15.80 ± 1.1

Cytosols were prepared as described in Experimental and incubated with 10^{-9} M [^{3}H]-estradiol for 2.5 h. Bound steroid was determined with Dextran-coated charcoal, as described. Specific binding was taken as the amount of [^{3}H]-estradiol displaced by a 1000-fold molar excess of non-radioactive estradiol.

* Normal hamster value is significantly different from all other values. $P \leq 0.01$

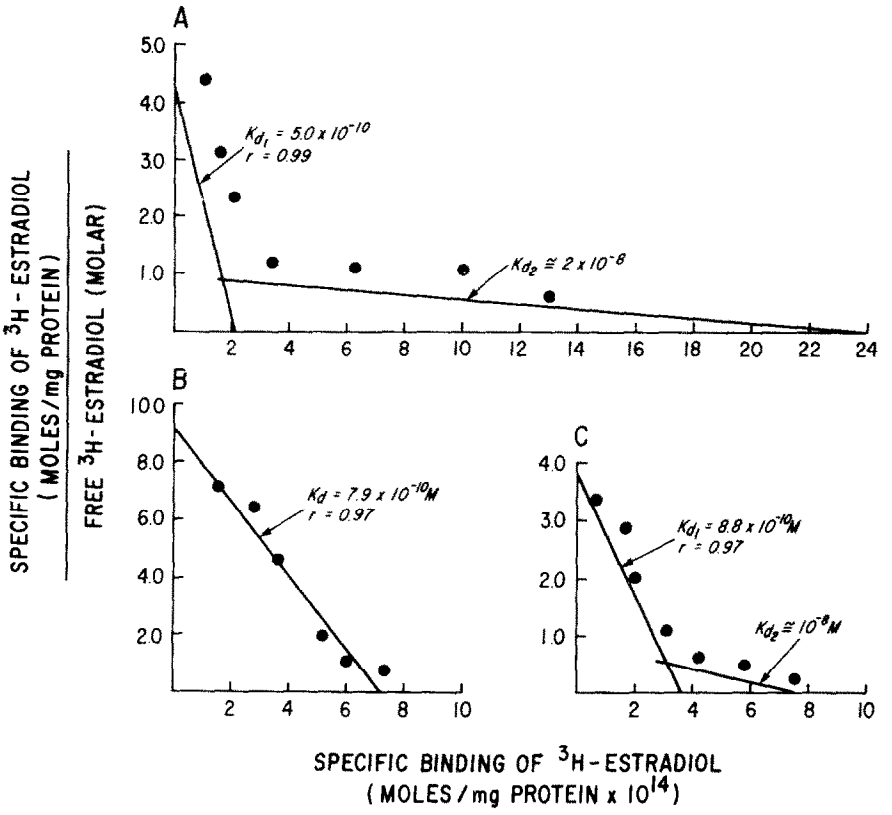

Fig. 3. Scatchard analysis of specific [^{3}H]-estradiol binding in renal cytosols from (A) a normal hamster, (B) a renal tumor bearing hamster and (C) a normal rat. Cytosols were incubated with [^{3}H]-E_2 over a 100-fold concentration (3×10^{-10} M to 3×10^{-8} M) for 2 h. Parallel incubations with a 1000-fold excess of nonradioactive estradiol were performed to determine nonspecific binding. The dissociation constant(s) for each *group* are presented in Table 2.

Nuclear accumulation of [^{3}H]-estradiol

The amount of specifically bound [^{3}H]-E_2 retained in the nuclear fraction after incubation of slices of tissues is presented in Table 3. As documented in this table, the highest amount of extractable [^{3}H]-E_2 was found in the nuclei prepared from slices of kidney from the normal Sprague–Dawley rat. Moreover, there was a minimum amount of variation ($15.83 \pm 1.1 \times 10^{-15}$ mol/mg protein). The amount of bound [^{3}H]-E_2 per mg protein in the normal hamster was not significantly different from that in tumor, as purified nuclei from normal hamster contained $3.28 \pm 0.96 \times 10^{-4}$ mol/mg protein ($N = 13$) and from TH animals contained $2.87 \pm 0.56 \times 10^{-14}$ mol/mg protein. However, when the DNA content of the purified nuclear pellet was quantitated and the specifically bound [^{3}H]-E_2 was expressed in terms of DNA rather than protein there was a significant difference between the normal and tumor animals. Nuclei derived from the normal hamster bound $3.19 \pm 0.63 \times 10^{-15}$ mol estradiol/mg DNA while nuclei from tumor hamster bound significantly more estradiol ($6.70 \pm 0.86 \times 10^{-15}$ mol/mg DNA).

DISCUSSION

In our studies we have demonstrated that (1) the cytosol and nuclear binding of [^{3}H]-estradiol in ham-

Table 2. Quantitation of higher affinity cytosol binding of [^{3}H]-estradiol

Source of cytosol	N	K_D^* (10^{-10} M)	N_{max}^* (10^{-14} mol/mg protein)
Normal hamster	7	5.05 ± 0.82	2.89 ± 0.26
Tumor hamster	6	7.44 ± 1.20	8.59 ± 1.59†
Normal rat	4	6.65 ± 1.38	8.37 ± 0.95†

* The apparent dissociation constant (K_D) and maximal number of binding sites (N_{max}) for the higher affinity component (in the case of normal animals) and of the only component in tumor hamsters were derived from the slope and intercept of Scatchard plots.

† Significantly different from normal hamster, $P = 0.001$.

Table 3. Specific nuclear binding of [^{3}H]-estradiol in renal slices

Source of tissue	Nuclear binding*	
	10^{-14} mol/mg protein	10^{-15} mol/mg DNA
Normal hamster	3.28 ± 0.96 (13)	3.19 0.63 (4)
Tumor hamster	2.87 ± 0.56 (15)	6.70 ± 0.86† (7)
Normal rat	21.80 ± 0.8† (6)	Not done

* Specific nuclear binding of [^{3}H]-estradiol was determined in slices of tissue, as described in Experimental.
† Significantly different from other values in same column, $P \leq 0.025$.

ster kidney increases with duration of treatment with E_2 (Table 1); (2) renal cytosols derived from normal hamsters and normal rats each have at least two classes of proteins which bind [^{3}H]-E_2, as characterized by differing affinities; (3) specific binding of [^{3}H]-E_2 in both NH and TH renal cytosols is displaceable by compounds with estrogenic activity and is not displaced by testosterone, corticosterone or progesterone; and (4) the kidney of the rat, a species which does not develop an estrogen-induced renal tumor, contains at least as much estrogen binder with comparably high affinity as does the kidney of normal hamster. These findings confirm and extend those of Li *et al.*[7].

Our finding of two classes of estrogen-binding sites in normal kidney (of both hamster and rat, Fig. 3), which contrasts with the single affinity binding of E_2 in the tumours, carries implications for the quantitation of estrogen binding. For example, if E_2 binding in normal hamster and tumor were conducted at an [^{3}H]-E_2 concentration of 10^{-8} M, one might find as much or more E_2 binding in the normal tissue, because of binding of the [^{3}H]-E_2 to the large capacity, low affinity component. Thus the importance of studying E_2 binding at several concentrations of hormone is emphasized. We are unable to state the biological or possible carcinogenic significance of this shift from two classes of binding sites in normal to a single affinity class in the tumor.

Our study of the estrogen binding abilities of the two species, only one of which develops the renal tumor, failed to demonstrate an unusually large quantity, or a unique affinity in the kidney of the species which develops the tumor. Indeed, the rat kidney binds at least as much E_2 (Tables 1 to 3, Fig. 1). In addition, the affinities of both the higher and lower affinity binding sites were comparable in the two species.

New data have been presented on the renal nuclear accumulation of [^{3}H]-E_2 both in the normal and tumor animals. The amount of extractable [^{3}H]-E_2 per mg DNA from purified nuclei of tumor animals was significantly higher than that seen in the normal hamster. This increase in nuclear accumulation of [^{3}H]-E_2 in the tumor animals corresponds with the evidence of increased cytosol binding of [^{3}H]-E_2 during development of the renal tumor. If one envisages the E_2 mediating its effect on renal tumorigenesis

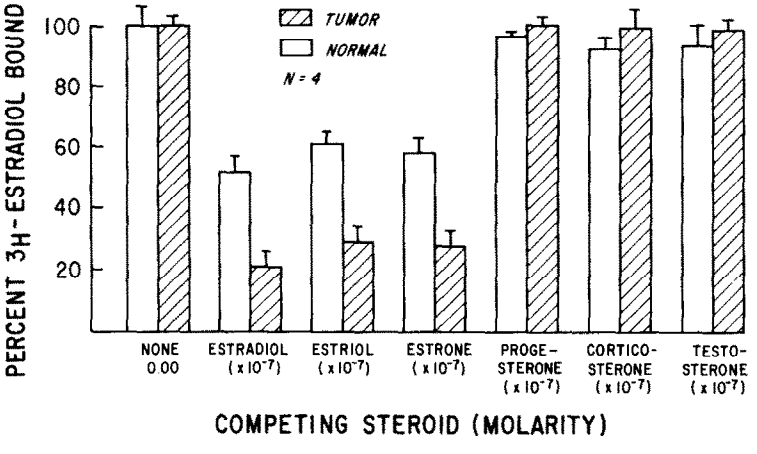

Fig. 4. The binding of [^{3}H]-estradiol in normal and renal tumor cytosols *in vitro*; competition by 10^{-7} M estradiol, estriol, estrone, progesterone, corticosterone and testosterone. Cytosols were incubated with 1.0×10^{-9} M E_2 ± nonradioactive steroid for 2 h and unbound steroid removed by dextran-coated charcoal. 100% binding represents the binding of [^{3}H]-E_2 in the absence of any nonradioactive steroid.

through a specific steroid receptor interaction, the relative amount of [^{3}H]-E_2 bound in the nucleus should be the most sensitive assay currently available for predicting a receptor mediated response. Of interest in this regard were the data which revealed no significant difference in nuclear binding of [^{3}H]-E_2 between the normal and tumor animals when binding was expressed in terms of extractable protein. However, as receptor bound [^{3}H]-E_2 in the nucleus is believed to interact directly with the genome, we believe that expressing bound [^{3}H]-E_2 per mg nuclear DNA more accurately reflects processes which may occur following translocation of the steroid–receptor complex to the nucleus.

The presence of specific [^{3}H]-E_2 binding proteins in the cytosol and nuclei of normal and tumor animals together with an increase in each of these parameters during tumorigenesis lends support to the concept of a receptor mediated response in renal tumorigenesis. However, as has been well documented in other tissues, notably the breast and prostate, tissues of many hormone-dependent malignancies are characterized by having the content of bound [^{3}H]-steroid increased by an order of magnitude or more[16, 17]. Thus, while our study is consistent with the concept of a receptor mediated response to E_2 during tumorigenesis, there is not a dramatic increase in steroid uptake by the tumor nuclei nor a receptor of unusual quantity or affinity in the species which develops the tumor. Therefore, other indirect actions of estradiol may be of equal or greater importance in the genesis of this renal tumor.

Acknowledgements—Research supported by NIH grant No. CA-15796. Fellowship support for Dr. Norman S. Anderson III by NIH grant No. CA-02837.

REFERENCES

1. Kirkham H.: Estrogen-induced tumors of the kidney in the Golden Syrian hamster. *Natn. Canc. Inst. Monogr.* **1** (1959) 1–59.
2. Llombart-Bosch A. and Peydro A.: Morphological, histochemical and ultrastructural observations of diethylstilbestrol-induced kidney tumors in the Golden Syrian hamster. *Eur. J. Cancer* **11** (1975) 403–412.
3. Hamilton J. M., Flaks A., Salvja P. G. and Maguire S.: Hormonally induced renal neoplasia in the male Golden Syrian hamster and the inhibitory effect of 2-Bromo-α-ergocryptine methanesulfonate. *J. Natn. Canc. Inst.* **54** (1975) 1385–1393.
4. Antonio P., Gabaldon M., Lacomba T. and Juan A.: Effect of the anti-estrogen nafoxidine on the occurrence of estrogen-dependent renal carcinoma. *Horm. Metab. Res.* **6** (1974) 522–524.
5. Steggles A. W. and King R. J. B.: The uptake of [6,7-^{3}H]-oestradiol by oestrogen dependent and independent hamster kidney tumors. *Eur. J. Cancer* **4** (1968) 395–401.
6. Steggles A. W. and King R. J. B.: Oestrogen receptors in hamster tumors. *Eur. J. Cancer* **8** (1972) 323–334.
7. Li J. J., Talley D. J., Li S. A. and Villee C. A.: An estrogen binding protein in the renal cytosol of the intact, castrated and estrogenized golden hamster. *Endocrinology* **96** (1974) 1106–1113.
8. Li J. J., Talley D. J., Li S. A. and Villee C. A.: Receptor characteristics of specific estrogen binding in the renal adenocarcinoma of the golden hamster. *Cancer Res.* **36** (1976) 1127–1132.
9. DeVries J. R., Ludens J. H. and Fanestil D. D.: Estradiol renal receptor molecules and estradiol-dependent antinatriuresis. *Kidney Intl.* **2** (1972) 95–100.
10. Anderson N. S. III, David Y. and Fanestil D. D.: Estrogen binding proteins in the kidneys of hamsters with estradiol induced renal carcinoma. *Fed. Proc.* **35** (1976) 568.
11. Anderson N. S. III and Fanestil D. D.: Corticoid receptors in rat brain: evidence for an aldosterone receptor. *Endocrinology* **98** (1976) 676–684.
12. Marver D., Goodman D. and Edelman I. S.: Relationship between renal cytoplasmic and nuclear aldosterone receptors. *Kidney Intl.* **1** (1972) 210–223.
13. Lowry O. H., Rosenbrough N. J., Farr A. L. and Randall R. J.: Protein measurements with folin phenol reagent. *J. biol. Chem.* **193** (1951) 265–275.
14. Burton K.: Study of the conditions and mechanism of the diephenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. *Biochem. J.* **62** (1956) 315–323.
15. Scatchard G.: The attraction of proteins for small molecules and ions. *Ann. N.Y. Acad. Sci.* **15** (1949) 660–672.
16. McGuire W. L. and Chamness G. C.: Studies on the estrogen receptor in breast cancer. In *Receptors for Reproductive Hormones* (Edited by B. W. O'Malley and A. R. Means). Plenum Press, New York London. Vol. 36 (1973) pp. 113–136.
17. Fergusson J. D.: The basis of endocrine therapy. In *Endocrine Therapy in Malignant Disease* (Edited by B. A. Stoll). W. B. Saunders, London (1972). pp. 237–246.