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High Yield of Primary Serially Transplanted Hamster Renal Carcinoma: Steroid Receptor and Morphologic Characteristics*

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Abstract—*Appreciable quantities* (10–20 g) *of transplanted estrogen-dependent hamster renal carcinoma can be consistently obtained after the* 3rd *serial transfer by i.p. injection of estrogen-induced primary renal tumor, either minced tissue or cell suspension, into* 3.0 month *DES or* 17β*-estradiol-treated hamster hosts. The latency period for transplant tumor growth was initially* 3.0 months *and about* 1.5 months *for successive serial transfers. The serially transplanted renal carcinoma contained five steroid hormone receptors similar to that found in the primary tumor in terms of such characteristics as cross-competition, affinity constant and sedimentation coefficient. Histologic characteristics of the serially transplanted renal tumor are also comparable to that observed in the primary carcinoma. However, the concentration of both estrogen and progesterone receptors are higher than previously reported values obtained for the primary renal tumor or its metastases.*

INTRODUCTION

THE ESTROGEN induced and dependent hamster renal carcinoma, initially reported by Kirkman and Bacon [1] and subsequently characterized histologically by Kirkman and others [2–4], represents an unusual steroid hormone dependent neoplasm. The recent demonstration [5] of considerable quantities of five specific steroid hormone receptors in this tumor is unique and provides a novel model for studying the interrelated actions of all steroid hormones and their respective receptors on tumor growth and inhibition. However, no extensive biochemical studies such as steroid receptor purification have been done because of the limited availability of tumor tissue. Although numerous reports have shown that this carcinoma is capable of being transplanted s.c. into hosts previously treated with DES [2, 4, 6, 7], the latency period for transplant growth of the primary renal carcinoma was prolonged (4–12 months) and the initial serial transplants were limited in size. Preliminary investigations in our laboratory confirmed these earlier findings. Transplanting primary renal tumors into the thorax, within the cheek pouch, and into the tail, has not improved either the frequency of successful renal tumor transplants or tumor yield [2, 8].

The present report characterizes steroid receptors in the transplanted primary renal tumor as well as receptors in serially transferred tumors. High tumor yields were obtained by i.p. injection into hamsters treated with DES for 3 months of either minced tumor preparations or renal tumor cells in suspension.

MATERIALS AND METHODS

Materials

17β(2,4,6,7-^{3}H) estradiol (105 Ci/mmole), (1,2,6,7-^{3}H) progesterone (103 Ci/mmole), (17α-methyl-^{3}H) R5020 (86 Ci/mmole), 5α(1,2,4,5,6,7-^{3}H) dihydrotestoterone(5α-DHT) (123 Ci/mmole), (6,7-^{3}H) triamcinolone acetonide(34 Ci/mmole), and (1,2,6,7-^{3}H) aldosterone (85 Ci/mmole) were provided by New England Nuclear, Boston, Mass. Ra-

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dioinert steroids* were obtained from either Sigma Chemical Co., St. Louis, Mo. or Calbiochem., San Diego, Calif. Trizma base, Norit A, dextran 80, dithiothreitol and all sucrose gradient standards used were supplied by Sigma. Ultrapure sucrose (RNase-free) was obtained from Schwartz/Mann, Orangeburg, N.Y. Eagle's minimal essential medium (MEM) was purchased from Grand Island Biological Co., N.Y. TED (10 mM Tris–HCl, 1.5 mM EDTA, 1 mM dithiothreitol, pH 7.4) buffer and TEDG (same as TED but containing 5–10% glycerol) buffer were used to prepare tumor cytosols.

Tumor induction and transplant

Primary renal carcinoma was induced in castrated male Syrian golden hamsters (LVG:LAK, outbred strain, Charles River Breeding Laboratories, Newfield, N.J.) following treatment of these animals with either diethylstilbestrol(DES) or 17β-estradiol for 8–11 months as described elsewhere in detail [5, 9, 10]. For tumor transplantation, primary renal tumors or its metastases were freed of necrotic or hemorrhagic areas and finely minced in MEM. Suspensions of tumor cells were prepared by passage of tumor fragments through a stainless steel screen (60 mesh) and washing twice in MEM containing 5% BSA. Cell viability (90–95%) was determined by trypan blue exclusion. Approximately 1.0–1.5 ml of resuspended minced tumor or isolated tumor cells ($5–20 \times 10^7$ cells) were injected i.p. into castrated animals previously treated with DES for 2.5–3.5 months. The latency period for the 1st tumor transfer was approximately 2.5–3.0 months and for succeeding serial transfers [2–5] between 1.5–2.0 months. In the receptor studies, all hormone pellets were removed from the host animals 48–65 hr prior to sacrifice to clear endogenous hormone. To characterize corticosteroid binders, hamsters bearing tumor transplants were adrenalectomized and maintained on 0.85% NaCl for at least 4 days prior to use.

Cytosol preparation

Transplanted renal tumors were initially homogenized in 2.5–3.0 vol of either TED buffer (for estrogen and androgen receptors) or TEDG buffer (for progesterone and adrenocorticoid receptors) and high speed cytosol fractions were prepared as desribed previously [5, 9–13]. Protein concentration of the filtered cytosols was determined by the method of Lowry *et al.* [14] using BSA as a standard.

Steroid receptor determinations

Cytosolic steroid receptor assays of the transplanted renal tumor samples were carried out as reported earlier using the dextran–charcoal method [5]. Cross competition studies were performed by the addition of non-labeled steroids, diluted as previously indicated [5], at 100-fold excess concentration immediately prior to the addition of labeled hormone. Scatchard analyses [15] for the steroid receptors in the transplanted tumor were carried out at tumor cytosol protein concentrations of 0.5–6.0 mg/ml. Details of the method used have been previously reported [9–12]. The concentration of steroid receptors in the renal carcinoma transplants was calculated from plots ($n=6–8$) varying protein concentration (0.1–13 mg/ml) versus specific hormone binding. Protamine sulphate precipitation was carried out according to the method of Steggles and King [16].

The procedure used for sucrose density gradient analyses and radioactivity measurements have been described elsewhere [5, 10–13].

RESULTS

Transplantation studies

Intraperitoneal injection of either minced primary renal tumor or renal tumor cell suspensions into 2.5–3.5 month DES-primed hamsters yielded 1–3 g of tumor per animal after 3 months of implantation. The tumor yield of the second serial transfer increased to approximately 5 g per animal after being implanted for 1.5–2.0 months. However, following the third serial i.p. transfer, the tumor yield was markedly augmented, generally to 10–15 g of tumor per animal and not uncommonly to 20 g of tumor tissue per hamster. (Fig. 1). As many as five serial transfers were carried out with undiminished tumor yields and unchanged latency period of 1.5 months. In 17β-estradiol treated hosts the frequency of successful transfers was nearly 100%. The tumors appeared as clusters of rounded white foci almost devoid of hemorrhagic or necrotic areas (Fig. 1). Preliminary studies indicate that i.p. serial renal tumor transfers did not take in animals pretreated for 2.7 months and

*The trivial names for synthetic steroids used are: R5020; 17,21-dimethyl-19-nor-4,9-pregnadiene-3,20 dione and triamcinolone acetonide, 9-fluoro-11β,21-dihydroxy-16α,17-1-methylethylidenebis(oxy)pregna-1,4-diene-3,20-dione.

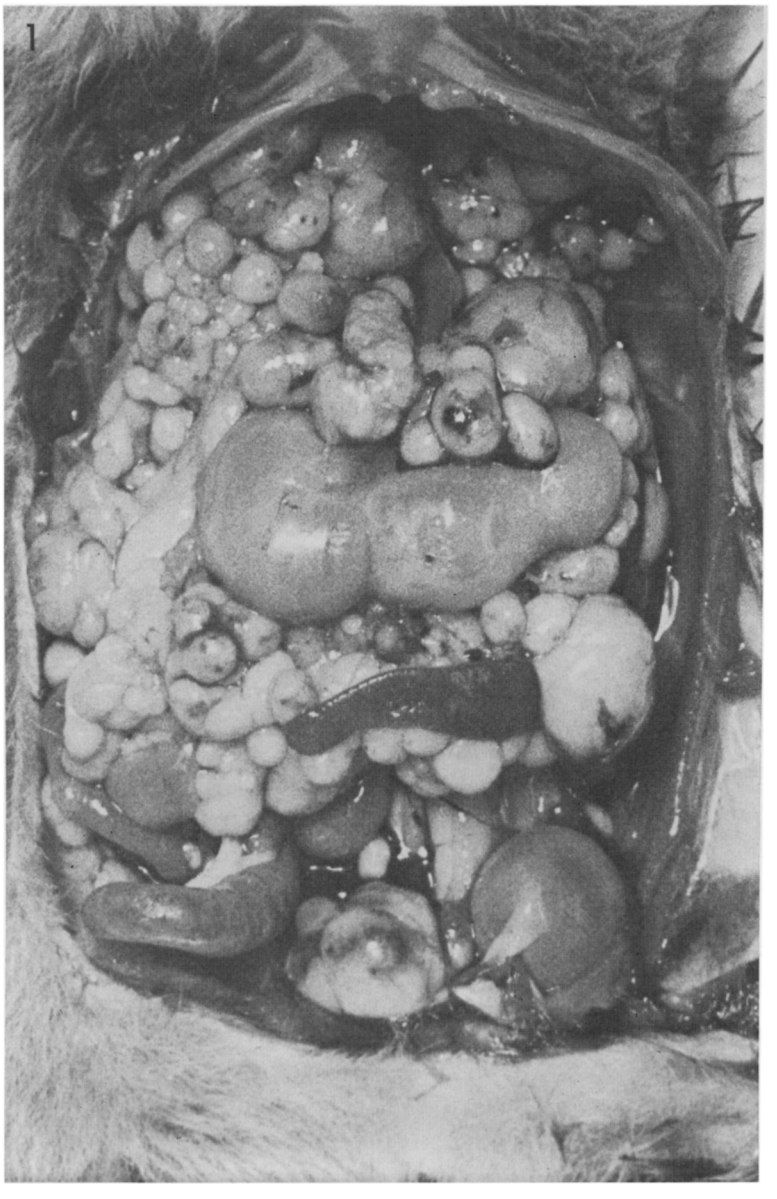

Fig. 1. *Ventral aspect of castrated male hamster opened to exposed multiple growing primary renal carcinomas in the third serial transfer. The animal had been treated with DES for* 3.5 months *prior to i.p. injection of* 1.0 ml *of minced tumor suspension. The hamster was sacrificed* 45 days *after implantation of the tumor suspension. Note the absence of necrotic or hemorrhagic areas in these tumors. The liver was removed to expose attached carcinomas. Tumor yield from this animal was approximately* 20 g.

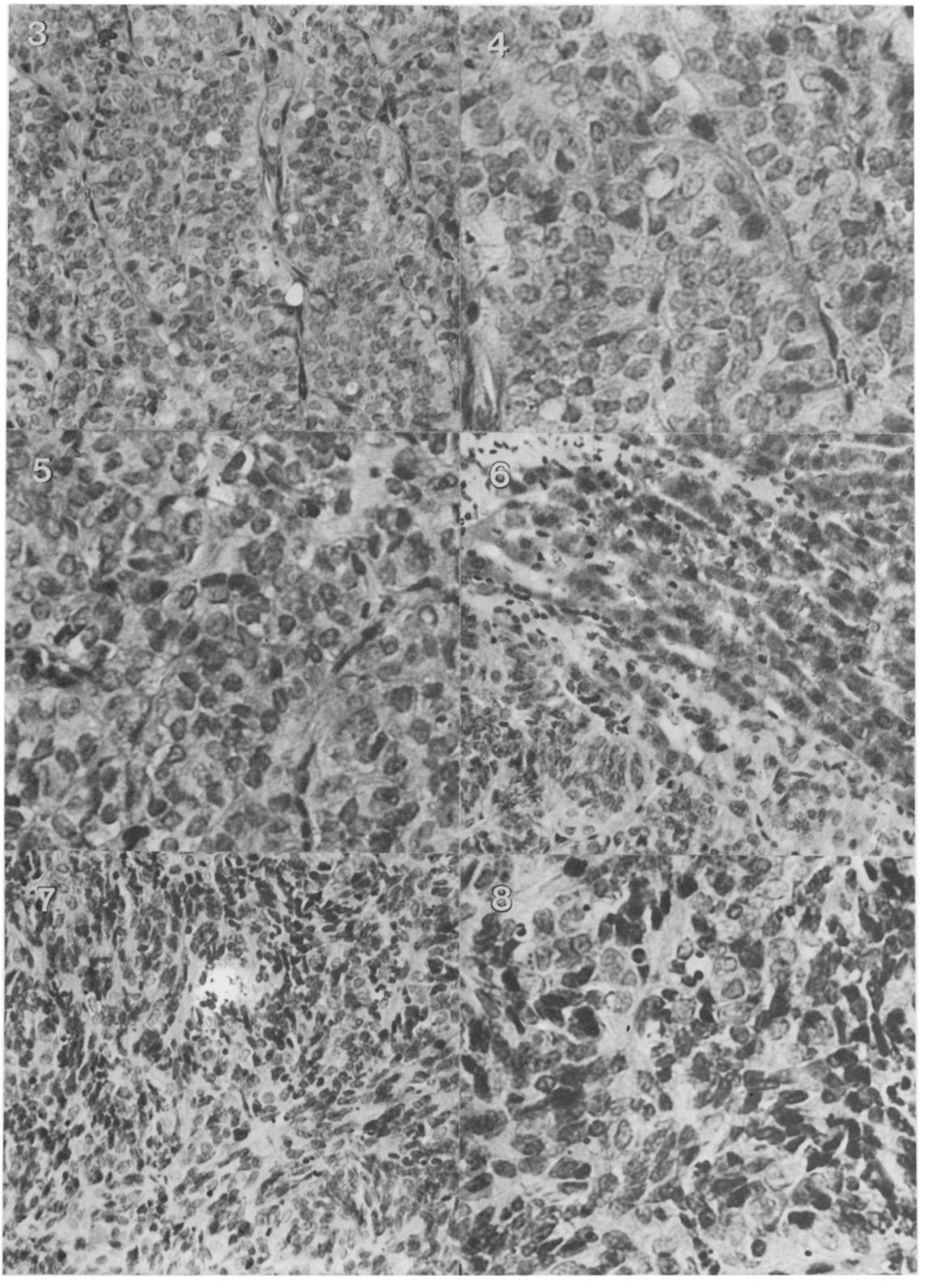

Fig. 3. *Renal tumor cells arranged in cords or plates from a first serial transfer of a renal tumor in a castrated male host implanted with DES for* 3.1 months. *Tumor implanted for* 95 days. *H* & *E.* × 40.

Fig. 4. *High magnification showing tubular arrangement in a first serial transfer of a renal tumor grown for* 95 days *in a castrated male host treated* 3.1 months *prior to implant with DES. H* & *E.* × 80.

Fig. 5. *Tubular formation in a second transfer of a renal tumor grown for* 45 days *in a castrated male host treated with DES for* 3.2 months *prior to implant. Tumor implanted for* 45 days. *Tumor cells are essentially cuboidal in shape and arranged in the characteristic pattern of a primary renal carcinoma. H* & *E.* × 80.

Fig. 6. *Two distinct essentially segregated cell types in a second transfer of a renal tumor grown for* 45 days *in a castrated male host treated with DES for* 3.2 months *prior to implant. H* & *E.* × 40.

Fig. 7. *Mixed cell types in a fourth serial transfer of a renal tumor grown for* 42 days *in a castrated male host prior to implant for* 3.3 months *with DES. H* & *E.* × 40.

Fig. 8. *Irregularly arranged columnar or spindale shaped cells in a fifth serial transfer of a renal tumor grown for* 42 days *in a castrated male host treated with DES for* 3.3 months *prior to implant. Note the variation in the intensity of nuclear*

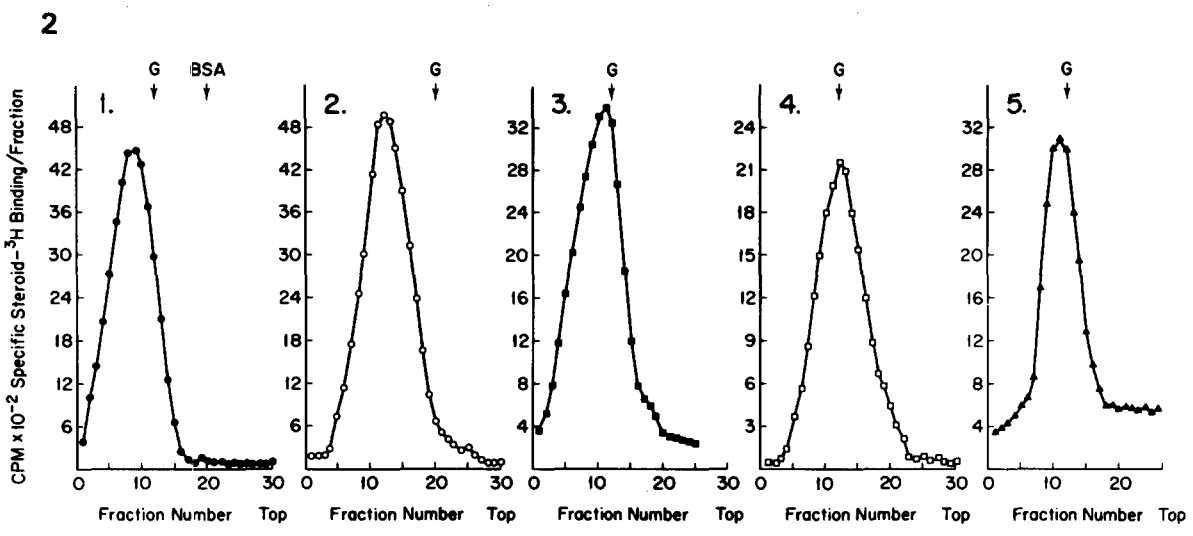

Fig. 2. Specific cytosolic steroid hormone binding in i.p. injected, serially transplanted hamster renal carcinoma. Tumor cytosols were incubated in vitro *with* 5 nM *tritiated steroid alone or in combination with unlabeled hormone at* 5×10^{-7} M(100×) *at* 0–3°C. *The cytosols were then treated with dextran–charcoal* (10–90 min) *to remove free and some lower affinity binding hormone. Aliquots of* 0.3 ml (0.5 ml *for aldosterone*) *were applied to each gradient containing specific* 1. *estradiol* (●), 2. *R*5020 (○), 3. 5α-*DHT* (■), 4. *triamcinolone* (□), *and* 5, *aldosterone* (▲), *binding. Gradient profiles represent specific hormone binding determined by subtracting profiles of the amount bound in the presence of* 100-*fold excess concentrations of radioinert hormone from profiles of the total binding obtained from incubations of radiolabelled hormone alone. Protein concentration in the cytosols was* 11.5±0.6 mg/ml. *Gamma globulin* (*G*) *and* ^{14}C-*bovine serum albumin* (*BSA*) *served as sedimentation coefficient standards.*

Table 1. *Steroid receptor characteristics in the serially transplanted hamster renal carcinoma*

Receptor	*S* values cytosol*	$K_A(10^9 M^{-1})$†	Receptor levels (fmole/mg cytosol protein‡)	Protamine sulphate (% ppt§)
Estradiol	8	2.2	321±53(7)	91
Progesterone	7	0.6	2721±336(7)	83
5α-DHT	8	1.3	179±15(7)	86
Triamcinolone	7	1.7	162±16(5)	78
Aldosterone	8	0.8	53±7(5)	76

*Sedimented in low salt medium. Sedimentation values were determined according to the method of Martin and Ames [17].

†Calculated from Scatchard plots at 0–3°C. Protein concentration range was 0.5–6 mg/ml. Data represent the average of 3 determinations.

‡Mean±S.E.M. The number of individual serial transfers (1–5) assayed is in parentheses.

§Data based on the average of 3 separate protamine sulfate precipitation (ppt) assays.

maintained on either 5α-DHT, progesterone or megestrol acetate. Similarly negative results were also obtained when the renal tumor was transferred into untreated intact males.

Steroid receptor analyses

Figure 2 and Table 1 summarize the results of the steroid receptor characteristics of the transplanted hamster renal carcinoma following i.p. injection. All steroid receptors in each of the serial transfers sedimented as either 8 S or 7 S moieties after centrifugation in low salt. Although infrequent, the presence of a specific 4 S estradiol binding component was observed in a few transplanted renal tumor cytosols. The relative concentration of these steroid receptors in the renal carcinoma are progesterone > estrogen > androgen > glucocorticoid > mineralocorticoid (Table 1). The initial two serial transfers had estrogen and progesterone receptor concentrations similar to levels found in the primary carcinoma and its metastases whereas the amount of these receptors in the

third through the fifth serial tumor transfers was approximately twice that found in the primary lesion. In contrast, the concentrations of androgen and adrenalocorticoid receptors were similar in all transfers made. Results of Scatchard analyses for each cytosolic steroid receptor in the transplanted renal tumors revealed high affinity binding and low capacity for their respective hormones.

Tumor transplant morphology

The histologic characteristics of the initial and second serial i.p. renal carcinoma transfers were similar (Figs. 3–5). In these transfers, the tumor cells are most commonly arranged in branching anastomosing cords and plates and cystic spaces are often seen. The majority of the renal tumor cells are either cubodial or columnar in shape (Figs. 4, 5). The morphology of the third and fourth serial transfers are essentially indistinguishable from the first two transfers and resemble the histologic characteristics of the primary carcinoma and its metastases [2]. The segregation of cell types (Fig. 6) in the fourth transfer has been previously reported by Kirkman [2] in both the primary lesion and in s.c. transplants. There is a tendency, however, for the renal tumor cells to become more irregular in shape with continued serial transfer (Figs. 7, 8) and nuclear clumping is common (Fig. 8). With continued i.p. serial transfer, the tumor cells exhibit more extensive areas of spindle-shaped cells which probably arise from metaplasia of epithelial cells as a result of prolonged serial transfer.

DISCUSSION

A variety of routes have been used serially to transfer primary renal tumors but none of these methods have yielded substantial quantities of renal carcinoma [2, 8]. Subcutaneous injection, the most commonly employed route used by other investigators in the transfer of renal tumors, results in necrotic or hemorrhagic tumors before they attain any appreciable size. The i.p. route, however, shortened the latency period for tumor transplant growth and allowed for marked proliferation of the neoplasm following the third and subsequent transfers. Although the serially transplanted renal carcinoma is similar to the primary tumor with respect to most parameters studied, an appreciable increase in both estrogen and progesterone receptor concentration was found in the transplanted tumor compared to levels found in the primary carcinoma [5]. This increase in both estrogen and progesterone receptor levels may be related in part to the consistently high quality of tumor tissue obtained which would be compatible with the slight elevation seen in the levels of the other steroid receptors. Additionally, the rise in the concentration of these receptors may also be due to a slight modification in the mechanisms under estrogenic control during transfers since the synthesis of both estrogen and progesterone receptors are affected whereas the levels of the other steroid receptors which are generally not considered to be under such control were not appreciably altered.

The i.p. serial transfers of the renal carcinoma resulting in high tumor yield and its general similarity to the primary tumor should expand the usefulness of this hormone-dependent tumor model to studies in which large amounts of renal carcinoma are required.

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