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CYTOSOL AND NUCLEAR ESTROGEN RECEPTOR IN THE GENITAL TRACT OF THE RHESUS MONKEY*

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SUMMARY

Estrogen receptor levels of cytosol and nuclei were measured in the cervix, vagina, myometrium, and endometrium of the rhesus monkey after the following experimental treatments: (1) castration; (2) castration with estradiol treatment; and (3) castration with estradiol plus progesterone treatment. Plasma hormone levels similar to those of normal menstrual cycles were achieved by implantation of silastic capsules containing estradiol or progesterone. The K_D, 4×10^{-9} M, for the cytosol receptor was similar in all tissues. Competition studies showed cytosol receptor affinities to be $E_2 > E_1 > E_3 >$ DES. Cytosol receptor levels in estrogenized animals were lowered by progesterone treatment. Progesterone treatment did not alter the levels of nuclear receptor indicating that the lower levels of cytosol receptor obtained with progesterone were not due to nuclear translocation of cytosol receptor. Estrogen treatment changed the sedimentation coefficient of the endomentrial and myometrial cytosol receptor from 4 *s* to 8 *s* when compared with the castrate. This alteration in sedimentation coefficient was blocked by progesterone treatment. Changes in sedimentation coefficient were not observed in the cervix or vagina. Nuclear receptor in estrogen and estrogen plus progesterone treated animals sedimented as a 5 *s* protein. The data suggest that both qualitative and quantitative changes in the estradiol cytosol receptor of the genital tract are induced by progesterone and that these alterations may be related to the hormone interactions in target organs.

INTRODUCTION

Effects of estrogen on target tissues are mediated through the binding of estrogen to a cytoplasmic receptor protein and translocation of this complex to the cell nucleus [1, 2]. The sedimentation coefficients of the nuclear and cytosolic estrogen receptors are distinctly different [3]. The sedimentation rate of the cytoplasmic receptor may be altered by changes of temperature [4] or ionic strength [5], and may be accomplished without the presence of nuclei [6].

Progesterone is generally considered to be antagonistic to the action of estrogen. It would seem reasonable that progesterone may cause a change in the binding properties or levels of estrogen receptors in the cytosol or nucleus, thereby opposing the action of estrogen. Hsueh *et al.*[7] have reported a decrease in the number of estrogen receptor sites with progesterone treatment in both the cytosol and nuclei of rat uteri.

This study describes the influence of estrogen and progesterone on the levels and sedimentation properties of cytosol and nuclei in several genital tract tissues of the rhesus monkey.

MATERIALS AND METHODS

[6,7-^{3}H]-estradiol-17β (S.A. 46.6 Ci/mmol) was purchased from New England Nuclear, and its purity was checked by Sephadex LH-20 column chromatography. Estradiol-17β and progesterone were obtained from Schwarz–Mann. Scintillation fluid was prepared by dissolving 5 g of Omnifluor (New England Nuclear) in 900 ml of toluene plus 100 ml of BioSolv (BBS3, Beckman).

Twelve adult rhesus monkeys were ovariectomized 4–6 weeks prior to use. The animals were divided into 3 equal groups. The first group was sacrificed 2 weeks after receiving silastic capsules containing estradiol. In the second group, implantation of estradiol was followed 2 weeks later by an implant of progesterone for 1 week before sacrifice. The third group were implanted with empty capsules 2 weeks prior to sacrifice. The capsules containing crystalline steroids were placed subcutaneously in the interscapular region of the back. The capsules were of the same dimensions as described by Karsch *et al.*[8]. Blood samples were drawn twice weekly during the experimental period for radioimmunoassay of plasma estradiol and progesterone.

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Animals, anesthesized with Sernylan, were sacrificed by exsanguination. The genital tract was removed and placed in cold isotonic saline. All procedures were carried out at 4°C unless otherwise noted. The tissues were blotted and weighed. The minced tissue was homogenized in 6 vol. (w/v) of TE buffer (10 nM Tris–HCl, 1.5 nM EDTA, pH 7.4, at 4°C) with 3 five-second bursts of a Polytron homogenizer. Homogenates were centrifuged at 800 *g* for 15 min. This supernatant was centrifuged at 105,000 *g* for 1 h on a Beckman ultracentrifuge model L3-50 with a type 50 fixed angle rotor.

The resultant supernatant or cytosol was vortexed with a pellet of dextran coated charcoal. Pellets were obtained from a suspension of dextran coated charcoal (250 mg/100 ml charcoal and 25 mg/100 ml dextran) equivalent in vol. to that of the cytosol. The mixture was incubated 15 min and centrifuged at 105,000 *g* for 20 min. An aliquot of the charcoal treated cytosol was used for the determination of the protein concentration by the procedure of Lowry *et al.*[9].

The assay of estradiol binding in cytosol was carried out as described previously [10]. In brief, 0.1 ml aliquots of cytosol were mixed with an equal vol. of TE buffer containing 0.1% gelatin and different concentrations of [^{3}H]-estradiol ranging from 1×10^{-10} to 2×10^{-8} M. Following incubation at 4°C for 24 h, 0.5 ml of dextran coated charcoal was added to each tube.

The mixture was vortexed and allowed to stand for 15 minutes. The tubes were centrifuged at 1500 *g* for 5 min. The supernatant solutions were decanted into scintillation vials and counted. Blank values were obtained by incubating a series of tubes containing the same concentrations of tracer in which the cytosol was replaced by 0.1 ml of buffer. These blank values were subtracted from the radioactivity in the supernatant of the corresponding assay tubes. Estimation of the dissociation constant and receptor site concentrations were made from Scatchard plots [11]. Statistical analyses were performed by Student's *t* test.

Competition study. Myometrial cytosol (0.1 ml) was incubated with 0.2 ml TE buffer containing 2,000 d.p.m. [^{3}H]-estradiol-17β (0.15 nM) and up to 100-fold excess of unlabelled competitor for 16 h at 4°C.

Sucrose gradient analysis of estradiol binding in cytosol. An aliquot of cytosol (0.2 ml) was incubated with approximately 30,000 c.p.m. (5 nM) [^{3}H]-estradiol with and without 100-fold excess estradiol for 24 h at 4°C and then layer layered onto 5 ml of a 5–20% sucrose gradient made in TE buffer. These gradients were centrifuged at 105,000 *g* for 16 h at 4°C in a Beckman L3-50 centrifuge with a SW 50.1 rotor. The gradient tubes received [^{14}C]-labelled bovine serum albumin and [^{14}C]-labelled rabbit gamma globulin as markers. The bottom of each tube was pierced and 8 drop fractions were collected for scintillation counting.

Determination of nuclear binding sites. The 800 *g* pellet from the tissue homogenate was resuspended in 10 vol. of TE buffer and rehomogenized in an all glass motor driven homogenizer. This nuclear suspension was centrifuged at 800 *g* for 10 min. The pellet was washed twice more with 20 vol. of TE buffer and centrifuged each time at 800 *g* for 10 min. The final nuclear pellet was homogenized with TE buffer with a few strokes of a glass homogenizer to provide a nuclear suspension equivalent to 20 mg of original wet weight of tissue per ml of buffer.

This suspension was then subjected to the estradiol-exchange assay of Anderson *et al.*[12], with adaptations as described by Somjen *et al.*[13]. Aliquots of suspended nuclei (0.5 ml) were placed into a series of paired tubes containing 5 nM, 10 nM, and 20 nM [^{3}H]-estradiol respectively in 0.2 ml of TE buffer. The first tube of each pair contained the tracer only, while the second tube contained the tracer plus 100-fold excess unlabelled diethylstilbesterol. The tubes were incubated with shaking for 1 h at 37°C. After incubation, 2 ml of TE buffer was added to each tube and they were centrifuged at 800 *g* for 10 min. The supernatant was discarded. The pellets were washed 3 more times with 3 ml of TE buffer. After the final wash, the pellets were dissolved in 0.4 ml of 0.3 M NaOH and allowed to stand at room temperature for 30 min. Aliquots (0.2 ml) of the solution in each tube were taken for counting. The remainder of the nuclear suspension was used for DNA determination [14].

The total specifically bound [^{3}H]-estradiol is the difference between the counts obtained in samples containing [^{3}H]-estradiol alone and those containing [^{3}H]-estradiol plus 100-fold excess diethylstilbesterol. Saturation of nuclear binding sites was found to occur at the 15 nM level. All subsequent calculations are based on the total number of specific binding sites at the 15 nM level and are expressed as pmol bound estradiol per mg DNA.

Sucrose gradient analysis of estradiol nuclear binding. Approximately 200 mg of tissue was homogenized in STE buffer (0.25 M sucrose 10 nM Tris–HCl, 1.5 nM EDTA, pH 7.4 at 4°C). The pellets were washed 3 times with 10 vol. of STE buffer. The washed pellets were suspended in 1.5 ml of STE buffer and incubated 1 h at 37°C in a 30 nM solution of [^{3}H]-estradiol. The suspensions were centrifuged at 1500 *g* for 10 min. The pellets were washed once with 0.4 ml of STE buffer and resuspended in 0.4 ml of 0.4 M KCl for 1 h at 4°C, after which the tubes were centrifuged at 1500 *g* for 10 min. The supernatant was vortexed with a charcoal pellet made from 0.4 ml of 250 mg/100 ml charcoal and 25 mg/100 ml dextran in TE buffer and incubated 15 min at 4°C. The dextran coated charcoal was removed by centrifugation at 1500 *g* for 5 min. The supernatant was centrifuged for an additional 30 min at 105,000 *g* and 0.2 ml of the resulting solution was applied to 5–20% sucrose gradients made in 0.4 M KCl and centrifuged at 105,000 *g* for 16 h in a SW 50.1 rotor. Eight drop fractions were collected and counted.

Table 1. Estradiol receptor concentrations in the cytosol of the genital tract of the Rhesus monkey

Tissue	fmol Bound E_2/mg Protein* C	E†	E + P
Vagina	189 ± 18‡	125 ± 35‡'§	135 ± 13§
Cervix	212 ± 34‡	174 ± 16‡	97 ± 17§
Myometrium	358 ± 75‡	220 ± 34‡	74 ± 22§
Endometrium	—	271 ± 48‡	113 ± 17§

* = Mean ± S.D. E† = Estrogen treated; E + P = Estrogen plus progesterone treated; C = Castrate. ‡'§ = Statistical analyses are for the individual tissues under the three hormonal states. Those treatments with differing superscripts are significantly different ($P < 0.05$).

RESULTS

The plasma concentration of estradiol in the estrogen and estrogen plus progesterone treated animals was not different and ranged from 80–100 pg/ml. The progesterone concentration in the latter group of animals was 5–7 ng/ml. In the nontreated ovariectomized animals, the levels of estradiol and progesterone were 20 pg/ml and 0.4 ng/ml respectively.

Hormone treatment alters the levels of estradiol receptor in the cytosol of genital tract tissues (Table 1). In all tissues examined there was no difference between castrate and those treated with estrogen. When estrogen and estrogen plus progesterone treated animals were compared, the level in the latter is significantly lower ($P < 0.05$) in all tissues except the vagina. The dissociation constant of the cytosol receptor is 4×10^{-9} M and is similar in all tissues.

Estrogen treatment significantly ($P < 0.05$) increases the levels of nuclear receptor in the vagina and cervix. A similar change is not found in the nuclear receptor levels of the myometrium (Table 2), but agreement amongst the four animals is poor. Castrated animals did not provide sufficient endometrial tissue for the measurement of receptor. Progesterone treatment of estrogenized animals does not significantly alter the levels of nuclear receptor in any of the genital tissues (Table 2), and again in this treatment group nuclei from cervix and endometrium show considerable variation.

The affinity of the myometrial cytosol receptor for estrogens is shown in Fig. 1. Binding of [^{3}H]-estradiol is reduced by 50% by a 10-fold excess of unlabelled estradiol, but the binding is reduced to a much smaller extent by the other 3 estrogens. At 100-fold excess of competitor, all 4 steroids reduce the binding by 50% or more. The order of affinities is seen to be $E_2 > E_1 > E_3 >$ DES. These data are similar to those previously reported in rabbit fallopian tube cytosol [15].

When the cytosol of genital tissue of the castrate animals was analyzed by sucrose gradients, a consistent 4 *s* peak which was displaced by excess estradiol, is found in all tissues (Fig. 2). In the estrogen treated animals, the sedimentation coefficient is not the same in all tissues (Fig. 3). The endometrium and myometrium have their peak activity in the 8 *s* region, while activity in the cervix peaks at 4 *s*. In the vagina, a

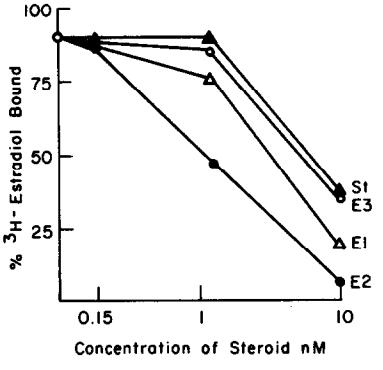

Fig. 1. Competition of unlabeled estrogens for myometrial cytosol receptor sites. E_2 = estradiol; E_1 = estrone; E_3 = estriol; St = diethylstilbestrol. A ten-fold excess of unlabeled estradiol reduces binding of [^{3}H]-estradiol by 50%. At 100-fold excess of competitor, all four steroids reduced binding by > 50%.

Table 2. Estradiol nuclear receptor concentrations in the genital tract of the Rhesus monkey

Tissue	pmol bound estradiol/mg DNA* C	E†	E + P
Vagina	8.2 ± 4.2‡	22.8 ± 3.7§	37.6 ± 4.9§
Cervix	16.0 ± 7.6‡	45.6 ± 9.0§	50.6 ± 21.4‡'§
Myometrium	14.4 ± 5.7‡	41.8 ± 15.9‡	12.8 ± 2.0‡
Endometrium	—	20.8 ± 9.2‡	26.8 ± 20.0‡

* = Mean ± S.E. E† —See Table 1, ‡'§ —See Table 1.

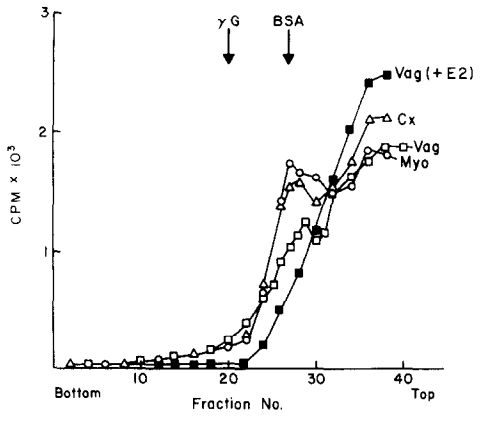

Fig. 2. Sucrose gradient analyses of [^{3}H]-estradiol binding in cytosols of genital tract tissues of ovariectomized monkeys. Vag = vagina; Cx = cervix; Myo = myometrium; E_2 = displacement of bound [^{3}H]-estradiol by 100-fold excess unlabeled estradiol. Arrows indicate peaks of ^{14}C labeled rabbit γ globulin (7 *s*) and ^{14}C labeled BSA (4 *s*). Displacement of bound [^{3}H]-estradiol by E_2, similar to that seen in the vaginal cytosol, was seen in myometrium and cervix.

small 8 *s* peak is found together with a larger 4 *s* component. Radioactivity in both the 4 *s* and 8 *s* regions (not illustrated) is displaced by excess estradiol indicating specific binding in both regions. Progesterone treatment abolishes binding in the 8 *s* region of both myometrium and endometrium. Peaks of activity in all tissues are again in the 4 *s* area as they are in the castrate state (Fig. 4), and bound radioactivity is displaced by "cold" estradiol.

Sucrose gradients of nuclear extracts show bound estradiol sedimenting in the 5 *s* region. This 5 *s* peak was seen in all tissues of estrogen − and estrogen +

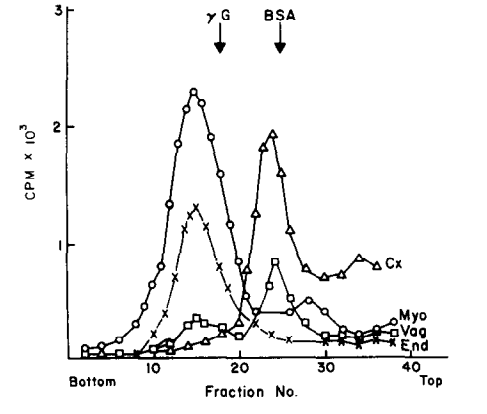

Fig. 3. Sucrose gradient analyses of [^{3}H]-estradiol binding in cytosols of genital tract tissues of estrogen treated monkeys. End = endometrium. Displacement of bound [^{3}H]-estradiol by excess unlabeled estradiol (not shown) occurred in both the 4 *s* and 8 *s* regions of all tissues where peaks were observed. Cytosols were treated with a dextran-coated charcoal pellet to remove unbound [^{3}H]-estradiol prior to placing placing cytosols on the sucrose gradients. For further explanation, see Fig. 2.

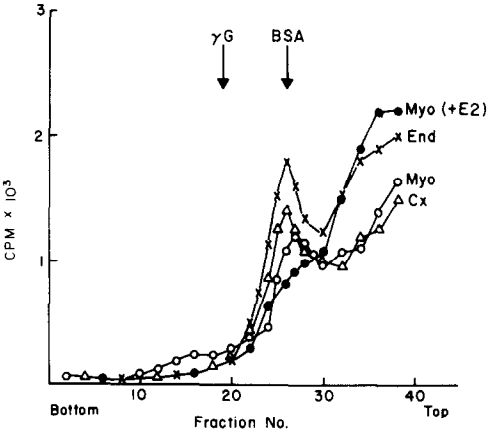

Fig. 4. Sucrose gradient analyses of [^{3}H]-estradiol binding in cytosols of genital tract tissues of estrogen plus progesterone treated monkeys. Displacement of [^{3}H]-estradiol by excess estradiol, as illustrated for myometrium, occurred in all tissues. See Figs. 2 and 3 for further details.

progesterone-treated animals. Gradients from an estrogen-treated animal (Fig. 5) illustrate the typical findings. Binding was highest in endometrial samples followed by myometrium while vagina and cervix showed much smaller amounts of bound radioactivity sometimes confined to a "shoulder" in the 5 *s* region of the gradient (Fig. 5).

DISCUSSION

In the endometrium, myometrium, and cervix of the rhesus monkey, a decrease in the levels of estradiol receptor of cytosol is associated with increased plasma levels of progesterone. Similar observations have been made in uterine tissue of other species [7, 16]. The vagina differs from the other genital tract tissues in that progesterone does not alter the level of receptor in cytosol. Hseuh *et al.*[7] have

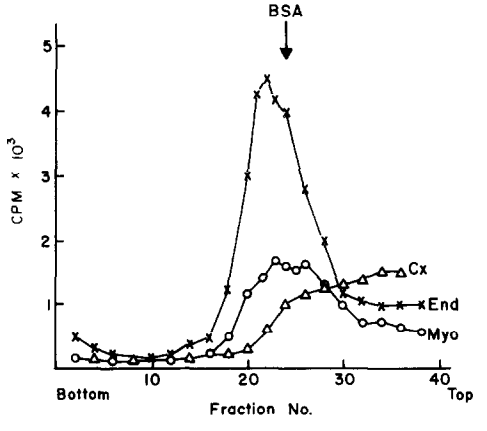

Fig. 5. Sucrose gradient analyses of bound [^{3}H]-estradiol extracted from nuclei of genital tract tissues of estrogen treated monkeys. Similar peaks were also observed for estrogen plus progesterone treated animals. See Figs. 2 and 3 for further details.

reported a depression in estradiol binding sites in both cytosol and nuclei of immature rat uteri under the influence of progesterone. In all of the genital tract tissues of the monkey, progesterone does not change the levels of nuclear receptor from those found in the estrogen treated animals. Thus the changes in cytosol receptor cannot be attributed to nuclear translocation of the cytosol estrogen receptor complex.

High levels of cytosolic estrogen receptor in the castrate when compared to estrogen treated animals may result from expressing the receptor concentration per mg protein. Martin *et al.*[17] have shown that although the absolute number of receptors are the same in castrate and estrogen treated rabbits, the relative amount of receptor per mg protein is higher in the castrate because the amount of protein in the cytoplasm of cells in castrate is lower than in estrogenized animals. However, expressing the results as receptor concentration per mg DNA did not alter the findings.

In the rhesus monkey, we have shown that estrogen treatment can induce a change in the sedimentation coefficient of the cytosol receptor which is reversed by treatment with progesterone. In the castrate, the estrogen–receptor complex in the cytosol sediments in the 4 *s* region for all tissues studied. With estrogen treatment, the sedimentation coefficient changes to 8 *s* in the endometrium and myometrium. Cytosol receptor preparations from the cervix and vagina of estrogenized animals maintain their sedimentation rate of 4 *s* with a small 8 *s* component in the vagina. The addition of progesterone causes a reversal of the effect of estrogen on the sedimentation rate of the estrogen receptor as evidenced by the fact that the receptor again sediments at 4 *s* in all tissues as it did in the castrate animal. Thus, it appears that the sedimentation coefficient of the estradiol cytosol receptor in the monkey differs in different organs and with different hormonal regimens. Those observations may explain the differences in sedimentation rates which have been reported for the uterus, 5 *s*, [18] and fallopian tube, 8 *s*, [16] in the monkey and for the cervix, 4.8 *s*, and endometrium, 5.2 *s* and 8.4 *s*, [19] in the human. Hunter and Jordan[20], working with human endometrium, have reported 4 *s* and 8 *s* binding components in the proliferative phase of the cycle, but only 4 *S* during the secretory phase of the cycle. In castrate or estrous rabbits, it has been noted that progesterone treatment does not alter sedimentation properties of the uterine cytosol receptor [17]. It is possible that the differences in sedimentation coefficients of this study reflect changes in the activity of proteolytic enzymes [21] or alterations of ionic and protein compositions of cytosol which are induced in specific organs by hormonal treatment.

These data provide new insight into the mechanism of hormone interaction, and suggest that interactions between estrogen and progesterone may be related primarily to quantitative and qualitative changes of the estrogen receptor of cytosol.

REFERENCES

1. Jensen E. V., and De Sombre E. R.: *Ann. Rev. Biochem.* **41** (1972) 203–230.
2. Jensen E. V., and De Sombre E. R.: *Science* **182** (1973) 126–134.
3. Jensen E. V., Brecher P. I., Mohla S. and De Sombre E. R.: *Acta Endocr., Copenh. Suppl.* **191** (1974) 159–172.
4. Jensen E. V., Suzuki T., Kawashima T., Stumpf W. E., Jungblut P. W. and De Sombre E. R.: *Proc. natn. Acad. Sci. U.S.A.* **59** (1968) 632–638.
5. Korenman S. G. and Rao B. R.: *Proc. natn. Acad. Sci. U.S.A.* **61** (1968) 1028–1033.
6. Jensen E. V., Numata M., Brecher P. I. and De Sombre E. R.: In *Biochemistry of Steroid Hormone Action*, Biochemical Society Symposia 32. (Edited by R. M. S. Smellie). Academic Press, London and New York, (1971) pp. 133–159.
7. Hsueh A. J. W., Peck Jr E. J. and Clark J. H.: *Nature* **254** (1975) 337–339.
8. Karsch F. J., Dierschke D. J., Weick R. F., Yamaji T., Hotchkiss J. and Knobil E.: *Endocrinology* **92** (1973) 799–804.
9. Lowry O. H., Rosebrough N. J., Farr A. L., Randall R. J.: *J. biol. Chem.* **193** (1951) 265–275.
10. Flickinger G. L., Muechler E. K. and Mikhail G.: *Fertil. Steril.* **25** (1974) 900–903.
11. Scatchard G.: *Ann. N. Y. Acad. Sci.* **51** (1949) 660–672.
12. Anderson J., Clark J. H. and Peck E. J.: *Biochem. J.* **126** (1972) 561–567.
13. Somjen D., Somjen G., King R. J. B., Kaye A. M. and Linder H. R.: *Biochem. J.* **136** (1973) 25–33.
14. Burton K.: *Biochem. J.* **62** (1956) 315–323.
15. Muechler E. K., Flickinger G. L., Mikhail G.: *Fertil. Steril.* **25** (1974) 893–899.
16. Brenner R. M., Resko J. A. and West N. B.: *Endocrinology* **95** (1974) 1094–1104.
17. Martin J. E., Philpott J., Chatkoff M., Young R. and Pauerstein C. J.: *Am. J. Obstet. Gynec.* **121** (1975) 180–186.
18. Wyss R. H., Karsznia R., Heinrichs W. L. and Hermann W. L.: *J. clin. Endocr. Metab.* **28** (1968) 1227–1230.
19. Sanborn B. M., Held B. and Kuo J. S.: *J. steroid Biochem.* **6** (1975) 1107–1112.
20. Hunter R. E. and Jordan V. C.: *J. Endocr.* **65** (1975) 457–459.
21. Notides A. C., Hamilton E. D. and Rudolph J. H.: *Biochim. biophys. Acta* **271** (1972) 214–224.