The relationships of the oestrogen and progesterin receptors in the abnormal uterus of the adult anovulatory rat

Effects of neonatal treatment with testosterone propionate or clomiphene citrate

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The neonatal administration of testosterone propionate to Wistar rats resulted in anovulatory adults in persistent vaginal oestrus. Clomiphene citrate had a similar effect. In both groups of adults, hyperplasia of the uterine epithelium and occasional metaplasia was observed. The uterine nuclear and cytosol oestrogen and progesterin receptors of these anovulatory rats were found to have affinities for their respective ligands similar to those of normal females. The nuclear oestrogen receptor comprised occupied and unoccupied components, as in normal females. The content of the nuclear oestrogen receptor was comparable with that of females in the late dioestrous or pro-oestrous phase. This content was higher in the clomiphene-treated group. Despite the relatively high nuclear oestrogen receptor content the content of progesterin receptors, a putative index of the oestrogenic response, was lower in the treated rats than in normal adult females throughout the cycle. Administration of oestradiol to both treatment groups resulted in depletion of cytosol oestrogen receptor content 1 h later, which, however, was not reflected by an increase in the content of nuclear oestrogen receptors. There was no measurable increase in progesterone receptor content in treated rats after daily administration of oestrogen (5 µg/rat) for 3 days. These changes in sex-hormone–receptor interactions involving an impairment of the normal oestrogenic response may be associated with the abnormal differentiation of the uterus in these sterile, anovulatory animals.

The actions of female sex hormones in target cells are mediated by their specific intracellular receptors; on hormonal stimulation these receptors are translocated from the cytoplasm into the nucleus where they are thought to regulate gene expression (Buller & O'Malley, 1976). The cellular response to oestradiol has been shown to be related to the concentration of nuclear oestrogen receptors (Anderson *et al.*, 1974), which varies according to the physiological state of the animal (Clark *et al.*, 1972; White *et al.*, 1978b; Myatt *et al.*, 1978). In the uterus oestradiol and progesterone are involved in regulating the cellular concentration of oestrogen receptors; oestradiol stimulation increases the concentration of its own receptor within 24 h and pretreatment with progesterone inhibits this process (Hsueh *et al.*, 1976). The concentration of progesterone receptors is increased by oestrogen and

decreased by progesterone stimulation (Vu Hai *et al.*, 1978; Kurl & Borthwick, 1979). This hormonal regulation of receptor concentrations appears to be fundamental to the normal physiology of the tissue. Progesterone antagonizes oestrogen-promoted epithelial proliferation, although it induced stromal proliferation (Finn & Martin, 1974). This epithelial effect has been related to a progesterone-modulated decrease in oestrogen receptor concentrations which in turn decreases tissue sensitivity to oestrogen stimulation (Coulson & Pavlik, 1977).

In the rat, plasma progesterone is derived from both ovarian and adrenal secretions (Feder *et al.*, 1968). Administration of androgens or oestrogens to neonatal female rats results in a failure of ovulation when they are adult. Progesterone secretion is not detectable from the ovaries of such animals (Sawada & Ichikawa, 1978), although oestradiol secretion is continuous and equivalent to that found in normal late dioestrous animals. We have used this

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anovulatory animal model to study the effects on the uterus of constant oestrogenic stimulation. The degree of cellular proliferation, together with the cellular content of both oestrogen and progesterone receptors, were measured as indices of the oestrogenic response. Such studies may lead to a better understanding of the effects of oestrogenic stimulation of the uterus, which occurs after the menopause, and of its therapy.

Experimental

Animals

All rats were bred in the experimental animal unit from a Wistar strain obtained from Charles River (U.K.) (Margate, Kent, U.K.). Female pups were selected and kept in groups of eight to ten animals, cross-fostered when necessary. Four to 5 days after birth pups were treated with one of the following: testosterone propionate (Sigma Chemical Co. Ltd., Poole Dorset, U.K.), 1.2 mg/animal subcutaneously in oil, or clomiphene citrate (Clomid: a gift we gratefully acknowledge from Merrel, Berks., U.K.), 500 µg/animal subcutaneously in oil; they were then returned to the lactating female. Animals were weaned at 20–21 days and housed as littermates with food and water available *ad libitum* with a 12 h dark/light cycle. At 120–140 days vaginal smears were taken on 4 consecutive days and those animals showing a persistent oestrus-like histology were selected for experimentation.

On the day of the experiment animals were used in groups of three and killed either before or 1 and 6 h after the administration of oestradiol-17β [2.5 µg/animal, intraperitoneally in 0.2 ml of 50% (v/v) ethanol/saline]. The ovaries were collected for histological examination. Uteri were weighed before removal of a longitudinal section of tissue for histological examination; the remainder was used for measurement of receptors. In the experiments where oestradiol was administered on 3 consecutive days the following procedure was followed: 5 µg of oestradiol-17β was injected subcutaneously in oil into each animal; on the fourth day animals were killed and each uterus was used separately for receptor measurements only.

Tissue was homogenized in Tris buffer containing 10 mM-Tris/HCl (pH 7.6)/1.5 mM-EDTA/1.5 mM-dithiothreitol/10% (v/v) glycerol (TEDG buffer) at 4°C in a type X10/20 homogenizer (Scientific Instrument Centre, London W.C.1, U.K.) with three 10 s periods of homogenization, alternating with 30 s cooling intervals. Pooled tissue was homogenized in 9 vol. (w/v) of buffer; single uteri were homogenized in 4 ml of buffer. A crude nuclear pellet was obtained by centrifugation of the homogenate at 1400 g for 10 min at 4°C. Further centrifugation of the supernatant at 200 000 g for 1 h at 4°C gave the cytosol

fraction. The nuclear pellet was washed three times by resuspension in the original volume of buffer used for homogenization. Each resuspension was followed by centrifugation at 1400 g for 10 min. The final pellet to be used for receptor assay was suspended in the original volume of buffer.

Histology

Both ovaries and a longitudinal strip of one horn of the uterus were taken from each animal and fixed in phosphate-buffered 10% (v/v) formalin. The tissues were embedded in paraffin wax before representative sections of both ovaries and serial sections of the uteri were cut and stained with Ehrlich's haematoxylin and eosin.

Measurement of oestrogen binding

Nuclear. Triplicate samples (0.2 ml) of the nuclear suspension were incubated at either (a) 30°C (to measure the total content of receptors, occupied plus unoccupied) or (b) 4°C (to measure the content of unoccupied receptors; Clark & Peck, 1979; Thrower & Lim, 1980; Levy *et al.*, 1980) for 1 h with radioactive oestradiol (15 nM; [2,4,6,7-³H]oestradiol-17β, 87–99 Ci/mmol; The Radiochemical Centre, Amersham, Bucks, U.K.) in the absence or presence of a 200-fold excess of diethylstilboestrol. In experiments to determine the dissociation constant of receptor–steroid interaction a range of steroid concentrations was used (0.1–30 nM). After three washes with 1 ml of TEDG buffer containing 1% (w/v) bovine serum albumin and a final wash with 1 ml of TEDG buffer alone the nuclear material was extracted with 2 × 1 ml of ethanol and monitored for radioactivity in 10 ml of butyl-PBD {5-(4-biphenyl)-2-(4-*t*-butylphenyl)-1-oxa-3,4-diazole} (Kontron Intertechnique, St Albans, Herts., U.K.). Subtraction of the radioactivity bound in the presence of diethylstilboestrol (non-specific) from the total radioactivity (without diethylstilboestrol) gave a measure of specific oestradiol binding.

Cytosol. Duplicate samples (0.2 ml) of cytosol were incubated in 5 nM-[³H]oestradiol at 4°C for 18–24 h in the absence or presence of a 200-fold excess of diethylstilboestrol. A range of steroid concentrations (0.1–30 nM) was used in experiments to determine the dissociation constant of steroid–receptor interaction. Chromatography on columns of Sephadex LH-20 (10 cm × 0.8 cm) (Thrower *et al.*, 1976) separated the macromolecular bound radioactivity from that not bound. A portion (1 ml) of the excluded material was collected for monitoring of radioactivity in 3 ml of Unisolve 1 (Koch-Light Laboratories Ltd., Colnbrook, Bucks., U.K.). The difference in radioactivity measured in samples incubated in the presence and absence of diethylstilboestrol was used to determine the concentration of oestrogen receptors.

Measurement of progestin binding

Nuclear binding. Triplicate samples (0.2 ml) were incubated with 20 nM-[1,2,6,7-³H]progesterone (90–110 Ci/mmol) (The Radiochemical Centre, Amersham, Bucks., U.K.) at 4°C for 18–24 h as described by Walters & Clark (1978) in the absence or presence of a 100-fold excess of unlabelled progesterone. A range of steroid concentrations (0.1–30 nM) was used in experiments to determine the dissociation constant. Cortisol (Sigma) at a 100-fold excess was included in each assay tube to minimize the binding of radioactive progesterone to the corticosteroid binding globulin. The washing procedure incorporated the modification suggested by Vu Hai *et al.* (1978), which involved (a) one wash in TEDG buffer containing 1% (w/v) bovine serum albumin and 0.2% Triton X-100, (b) three subsequent washes in TEDG buffer containing 1% (w/v) bovine serum albumin and (c) a final wash with TEDG buffer alone. Radioactivity in the final pellet was extracted and monitored as described for the oestrogen nuclear receptor. The difference in the amount of radioactivity measured in the presence and absence of unlabelled progesterone was used to calculate the concentration of nuclear progesterone receptor.

Cytosol. Duplicate samples (0.2 ml) were incubated with 20 nM-[³H]progesterone at 4°C for 18–24 h; cortisol and unlabelled progesterone were included as described above for the nuclear progestin binding. A range of steroid concentrations (0.1–30 nM) was used in experiments to determine the dissociation constant. The chromatography on columns of Sephadex LH-20 and monitoring of the radioactivity in these samples was identical with that described for oestrogen cytosol binding.

Monitoring of radioactivity

Radioactivity was monitored with an SL 3000 liquid scintillation counter (Kontron Inter technique, St Albans, Herts., U.K.) at a constant efficiency of 40%.

Results

Histology of reproductive tissues

Ovaries. In all of the treated animals the histology was abnormal compared with that of normal controls (Fig. 1). There was an increase in the number of follicles with all of the more mature follicles becoming cystic. Both primary and secondary follicles were present in fewer numbers than normal, especially in the clomiphene-treated animals. Corpora lutea were absent in the majority of animals and when present they were found to be regressed.

Uterus. There was a general thinning of the myometrium in all the treated animals (Fig. 2). The

spindle cell stroma is dense with numerous large glands in the pro-oestrus rat; all of the treated animals showed a decrease in the stromal cell content and gland size. There was also a general decrease in gland number, especially in the clomiphene-treated animals, although there was wide individual variation. The glandular cells were regular in basal nuclei arrangement, although in some of the testosterone-treated animals they showed an increased proliferation.

The endometrial epithelium of testosterone-treated animals displayed increased mitosis and cell proliferation along most of the length of the uterine lining. The clomiphene-treated animals showed a more pronounced cell proliferation, and in five animals metaplasia was seen along the lumen wall. Areas of endometrial erosion along the uterine wall were seen in these animals; this erosion in conjunction with the proliferating epithelium and associated metaplasia may be indicative of a pre-malignant condition (Novak & Woodruff, 1974).

Measurement of oestrogen and progestin receptors in androgenized rat uteri

Specific labelling of oestrogen nuclear receptors was maximal within 1–2 h at 30°C (Fig. 3a) and remained constant for up to 6 h. A similar time course was obtained when the incubation was performed at 4°C; the relatively rapid initial rate and plateau of labelling which was observed at this temperature suggested that measurement of receptors at 4°C was not the result of dissociation of endogenous bound ligand. In experiments where the initial 1 h period of labelling at 4°C was followed by prolonged incubation in the absence of labelled hormone for up to 6 h there was no evidence of dissociation of bound hormones (data not shown), confirming our previous observations in normal rats (Thrower *et al.*, 1981). The binding of [³H]-oestradiol to cytosol receptor at 4°C was maximal at 8 h and remained constant at 24 h (Fig. 3a).

Incubations of cytosol and nuclear fractions in the presence of [³H]progesterone for the measurement of progestin receptors were performed at 4°C (Fig. 3b). The time course of labelling was similar to that described by others for uterine progestin receptors (Clark & Peck, 1979).

Oestrogen receptor content and response to oestradiol

Oestrogen binding in the cytosol and nuclear fractions of both groups of neonatally treated animals was of high affinity. In the testosterone-treated group the dissociation constants (K_d) determined by Scatchard analysis (Scatchard, 1949) of the cytosol and nuclear binding were respectively 1.08 (1.42, 0.74) and 3.02 (2.96, 3.08) nM (Fig.

Fig. 1. *Histology of ovaries*

Transverse section ($\times 25$) of ovaries from 120–140-day-old (a) normal dioestrous female rats and anovulatory female rats treated neonatally with (b) testosterone propionate or (c) clomiphene citrate. Details of treatment are given in the Experimental section. Note the presence of multiple cystic follicles in (b) and (c).

Fig. 2. *Histology of uteri*

Longitudinal section ($\times 130$) of uteri from adult female rats, whose ovarian histology is shown in Fig. 1: (a) normal dioestrous, (b) testosterone-treated and (c) clomiphene-treated. Note hyperplasia and metaplasia of endometrial epithelium in (b) and (c).

Fig. 3. Effect of temperature and time on the specific binding of ^3H -labelled steroid to the nuclear and cytosol receptors of uteri from testosterone-treated rats

Uteri were collected from ten anovulatory rats, homogenized and then fractionated as described in the Experimental section. (a) Oestrogen receptors: triplicate samples (0.2 ml) of the nuclear preparation were incubated with 15 nM - ^3H -oestradiol in the presence and absence of a 200-fold excess of diethylstilboestrol, either at 30°C (○) or 4°C (●) for the indicated time periods before the determination of receptor content as described in the Experimental section. Duplicate samples (0.2 ml) of cytosol were incubated in 5 nM - ^3H -oestradiol in the presence and absence of a 200-fold excess of diethylstilboestrol at 4°C for the indicated time periods (Δ). (b) Progestin receptors: triplicate nuclear samples (0.2 ml) were incubated at 4°C in 20 nM - ^3H -progesterone, with a 100-fold excess of cortisol with and without 100-fold excess of unlabelled progesterone at 4°C for the indicated time periods before the determinations of receptor content. Duplicate cytosol samples (0.2 ml) were incubated at 4°C in a similar manner for the indicated time periods. ■, Nuclear; □, cytosol.

4a,b); those for the clomiphene-treated group were respectively 2.09 (0.65, 3.52) and 4.03 (3.70, 4.36) nM (Fig. 5a,b). The concentration of nuclear oestrogen receptors in the clomiphene-treated groups was significantly higher than in both the group treated with testosterone and the control animals (Table 1.)

One hour after the administration of oestradiol there was a significant decrease in the content of cytosol receptors in each treatment group (Table 1). Six hours after oestradiol injection the cytosol receptor content had not been completely replenished.

Despite cytosol receptor depletion there was no increase in the content of 'total' nuclear oestrogen receptors 1 h after oestradiol administration in either group. Six hours after administration the nuclear content remained unchanged in the testosterone-treated group. However, in the clomiphene-treated

group the corresponding content of receptors was significantly decreased.

Unoccupied nuclear oestrogen receptors

Unoccupied nuclear receptors were present in both the testosterone- and clomiphene-treated groups (Table 1); similar receptors are also present in the nuclei obtained from normal uteri (White *et al.*, 1978a; Thrower & Lim, 1980). The dissociation constant (K_d) of this binding was respectively 2.30 (2.08, 2.51) and 2.99 (1.54, 4.44) nM (see Figs. 4b and 5b). Although the content of unoccupied receptors was higher in the clomiphene-treated group, they were present in similar proportions of the total receptor in both treated groups. Oestradiol administration to the testosterone-treated group resulted in a decrease in unoccupied nuclear receptors 1 and 6 h later. Oestradiol administration to the clomiphene-

Fig. 4. Scatchard analysis of ^3H -labelled steroid binding to high-affinity receptors from uterine tissue of testosterone-treated rats

Samples (0.2 ml) of cytosol and nuclei were incubated with a range (0.1–30 nM) of concentrations of either ^3H oestradiol (a, b) or ^3H progesterone (c, d) with and without an excess of the appropriate competitors (see the Experimental section). Specific binding was calculated from the difference in radioactivity in the absence and presence of unlabelled steroid and plotted according to Scatchard (1949). (a) Cytosol oestrogen incubated at 4°C for 18–24 h. (b) Nuclear oestrogen incubated at 30°C (●) and 4°C (○) for 1 h. (c) Cytosol progesterone and (d) nuclear progesterone incubated at 4°C for 18–24 h.

treated group resulted in a decrease only after 6 h but this was not significant.

Progesterone binding

The dissociation constant (K_d) of the binding of progesterone to the cytosol and nuclear fractions from the testosterone-treated group (Fig. 4c,d) was 2.51 (2.66, 2.35) and 3.81 (2.82, 4.79) nM respectively. In the clomiphene-treated group the corresponding measurements of the K_d values were 5.52 (7.64, 3.40) and 5.58 (8.40, 2.75) nM respectively.

Fig. 5. Scatchard analysis of ^3H -labelled steroid binding to high-affinity receptors from uterine tissue of clomiphene-treated rats

Samples (0.2 ml) of cytosol and nuclei were treated as described for Fig. 4(a–d).

There was no significant difference between progesterone binding in the testosterone and clomiphene-treated groups; the cytosol content was respectively 4.01 ± 2.52 (testosterone) and 3.27 ± 2.51 (clomiphene) pmol/g of tissue ($n = 12$); corresponding nuclear concentrations were respectively 2.80 ± 1.04 and 5.50 ± 2.59 pmol/g of tissue ($n = 12$). There was no significant difference in the content of cytosol or nuclear progesterone receptor after oestradiol administration. The total receptor content (nuclear plus cytosol) in both the testosterone-treated group (6.93 ± 3.49 pmol/g of tissue, $n = 12$) and clomiphene-treated group (8.77 ± 2.98 pmol/g of tissue, $n = 12$) was significantly lower than that found in control animals from our colony at late dioestrus (15.70 ± 2.59 pmol/g of tissue) (Myatt *et al.*, 1980) and that reported by others (Vu Hai *et al.*, 1978; Kurl & Borthwick, 1979).

Effect of daily oestradiol administration

Subcutaneous injections of $5 \mu\text{g}$ of oestradiol were given daily for 3 days to determine whether this chronic treatment could induce the synthesis of progesterone receptor, as observed in normal tissue (Leavitt *et al.*, 1978; Kurl & Borthwick, 1979). Ovariectomy was considered unnecessary since each animal within the testosterone- or clomiphene-

Table 1. *Effects of oestradiol administration on the nuclear and cytosol oestrogen receptor content in uteri of neonatally treated anovulatory rats*

Adult anovulatory rats were separated into groups of three and uterine receptor content was measured either before or 1 and 6 h after the administration of 2.5 µg of oestradiol intraperitoneally (in 0.2 ml of ethanol/0.9% saline). Tissue preparation, fractionation and measurement of receptor were as described in the Experimental section; each value is the mean ± S.D. of four separate estimations, rats treated neonatally with either testosterone propionate or clomiphene citrate being used. Data from control animals are presented for comparison. Statistical analysis was by Student's *t* test: *significantly different from 0 h ($P < 0.01$); **significantly different from 0 h ($P < 0.001$); †significantly different from 0 h ($P < 0.005$); ††significantly different from 0 h ($P < 0.05$).

Time after injection of 2.5 µg of oestradiol (h) ...	Receptor (pmol/g)		
	0	1	6
Testosterone propionate			
Nuclear receptor measured by exchange assay	3.73 ± 0.95	3.88 ± 1.37	3.06 ± 2.30
Nuclear receptor measured at 4°C	2.10 ± 0.21	0.86 ± 0.26**	1.08 ± 0.06**
Cytosol receptor	3.17 ± 1.42	0.32 ± 0.25*	1.58 ± 1.00
Clomiphene citrate			
Nuclear receptor measured by exchange assay	11.35 ± 2.75	11.87 ± 5.10	2.19 ± 0.37†
Nuclear receptor measured at 4°C	4.83 ± 3.15	3.47 ± 2.44	1.85 ± 0.24
Cytosol receptor	4.95 ± 1.31	1.27 ± 0.66†	1.47 ± 0.17†
Control			
Nuclear receptor measured by exchange assay	2.98 ± 2.55	7.05 ± 1.55††	
Cytosol receptor	9.96 ± 2.35	1.85 ± 0.79**	

treated groups was under constant oestrogen stimulation. No increase in progesterone receptor content was observed. In our studies on the developmental aspects of postnatal androgenization, we have similarly found that oestrogen administration for 3 days to 3–4 week old rats did not lead to induction of progesterone receptors in neonatally treated females whereas in normal immature rats progesterone receptor content was increased approximately 2-fold (J. White, W. Marr, P. Moore, M. Elder & L. Lim, unpublished work).

Discussion

Studies on the effects of neonatal administration of testosterone to rats have clarified the regulatory role of sex steroids in hypothalamic differentiation (Gorski, 1971). Androgenized rats have defective hypothalamic functions; these may be related to abnormalities in oestrogen–receptor interactions (White & Lim, 1980). Androgenization also leads to peripheral effects, although the question of whether these may be mediated at the pituitary level is still unresolved. One such effect is an abnormal morphology in the uterus of adult rats (Lobl & Maenza, 1975), which is also manifest after neonatal administration of clomiphene, although the latter agent causes more widespread tissue abnormalities (Clark & McCormack, 1977). However, little is known about the interaction of the oestrogen receptor in the affected uterus. Uptake of radioactive oestradiol into the uterus is decreased in the androgenized animals (Vertes & King, 1971; Lobl, 1975) despite normal concentrations of cytosolic

oestrogen receptors (Lobl, 1975; Thrower *et al.*, 1978). The results of our present work demonstrate that there were profound changes, at the nuclear level, in the interaction of the oestrogen receptor in the uteri of the androgenized animals.

The presence of high concentrations of nuclear receptor in both treatment groups was consistent with a high level of oestrogenic stimulation of the uterus, as seen during late dioestrus and pro-oestrus. However, the high incidence of hyperplasia and metaplasia in the epithelial layer as well as a decreased stromal cell content in the uteri of the treated animals contrasted with normal uterine morphology at these phases (Fig. 2). These differences may well be related to the low concentration of progesterone in androgenized animals, since in the normal rat uterus progesterone suppresses epithelial but promotes stromal cell proliferation (for review see Martin, 1980).

An abnormal receptor response to oestrogen stimulation was associated with this altered histology. The administration of oestradiol resulted in considerable depletion of cytosol receptor, as normally seen, but not in an increase in nuclear receptor content. Furthermore, in the clomiphene-treated group, oestradiol stimulation surprisingly led to a decrease in nuclear receptor content within 6 h.

In the rodents the synthesis of uterine progesterone receptors is regulated by oestrogens; nuclear oestrogen receptors probably are involved in the interaction of transcriptional events leading to this synthesis (Leavitt *et al.*, 1978). Kurl & Borthwick (1979) have shown that the progesterone

receptor of the normal rat uterus is under oestrogenic control; after ovariectomy the cytosol progesterin receptor content fell to low values, which could be restored to higher levels after a single injection of oestradiol. Despite the high concentration of nuclear oestrogen receptors, the concentration of progesterone receptors was abnormally low in the uteri of both groups of neonatally treated anovulatory rats. Administration of oestradiol did not increase this concentration.

It is relevant at this stage to discuss the relationship of abnormal oestrogen responses to the cancerous state. Many tumours that contain oestrogen receptors fail to respond to endocrine therapy; this has been related to possible defects in the pathway of oestrogen stimulation which are beyond the initial receptor-binding steps. The loss of the ability to synthesize progesterone receptors has been also proposed to occur during the development of cancer of the uterus (Young *et al.*, 1976). The concentration of progesterone receptors was reported to vary with the degree of differentiation of the tumour (Pollow *et al.*, 1975), although other reports disagree (e.g. MacLaughlin & Richardson, 1976). Constant exposure to unopposed oestrogen is generally accepted to lead to neoplasia in several tissues and organs (Clark & Peck, 1979). In many cases this is accompanied by a loss of oestrogen dependence and many of the oestrogen-stimulated responses, including progesterone receptor synthesis.

The abnormal histology and abnormal oestrogen receptor responses of the uteri of the androgenized rats are thus probably the result of constant oestrogenic stimulation, unrelieved by progesterone. These androgenized rats therefore provide a most suitable animal model for studying the involvement of oestrogens in endometrial carcinoma and the ameliorative effect of progestins. We have since found that daily administration of progesterone to the androgenized animals restores normal uterine morphology and oestrogen-receptor interaction (J. White, P. Moore, M. Elder & L. Lim, unpublished observations).

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References

- Anderson, J. N., Peck, E. J., Jr. & Clark, J. H. (1974) *Endocrinology* **95**, 174–178
- Buller, R. E. & O'Malley, B. W. (1976) *Biochem. Pharmacol.* **25**, 1–12
- Clark, J. H. & McCormack, S. (1977) *Science* **197**, 164–165
- Clark, J. H. & Peck, E. J., Jr. (1979) *Female Sex Steroids: Receptors and Function*, p. 135, Springer-Verlag, Berlin, Heidelberg, New York
- Clark, J. H., Anderson, J. N. & Peck, E. J., Jr. (1972) *Science* **176**, 528–530
- Coulson, P. B. & Pavlik, E. J. (1977) *J. Steroid Biochem.* **8**, 205–212
- Feder, H. H., Resko, J. A. & Goy, R. W. (1968) *J. Endocrinol.* **41**, 563–569
- Finn, C. A. & Martin, L. (1974) *J. Reprod. Fertil.* **39**, 195–206
- Gorski, R. A. (1971) in *Frontiers of Neuroendocrinology* (Martini, L. & Ganong, W. F., eds.), pp. 237–290, Oxford University Press, New York and London
- Hsueh, A. J. W., Peck, E. J., Jr. & Clark, J. H. (1976) *Endocrinology* **98**, 438–444
- Kurl, R. N. & Borthwick, N. M. (1979) *J. Endocrinol.* **83**, 41–51
- Leavitt, W. W., Chen, T. J., Do, Y. S., Carlton, B. D. & Allen, T. C. (1978) in *Receptors and Hormone Action* (O'Malley, B. W. & Birnbaumer, L., eds.), pp. 157–188, Academic Press, New York
- Levy, C., Mortel, R., Eychenne, B., Robel, P. & Baulieu, E.-E. (1980) *Biochem. J.* **185**, 733–738
- Lobl, R. T. (1975) *J. Endocrinol.* **66**, 79–84
- Lobl, R. T. & Maenza, R. M. (1975) *Biol. Reprod.* **13**, 255–264
- MacLaughlin, D. T. & Richardson, G. S. (1976) *J. Clin. Endocrinol. Metab.* **42**, 667–678
- Martin, L. (1980) in *Estrogens in the Environment* (McLachlan, G., ed.), pp. 103–130, Elsevier/North-Holland, Amsterdam
- Myatt, L., Chaudhuri, G., Elder, M. G. & Lim, L. (1978) *Biochem. J.* **176**, 523–529
- Myatt, L., Elder, M. G. & Lim, L. (1980) *J. Endocrinol.* **87**, 365–373
- Novak, E. R. & Woodruff, J. D. (1974) *Novak's Gynaecologic and Obstetric Pathology with Clinical and Endocrine Relations*, 7th edn., p. 84–90, W. B. Sandler Co., Philadelphia, London and Toronto
- Pollow, K., Lübbert, H., Boquoi, E., Kreuzer, G. & Pollow, B. (1975) *Endocrinology* **96**, 319–328
- Sawada, T. & Ichikawa, S. (1978) *Endocrinology* **102**, 1436–1444
- Scatchard, G. (1949) *Ann. N.Y. Acad. Sci.* **51**, 660–672
- Thrower, S. & Lim, L. (1980) *Biochem. J.* **190**, 691–695
- Thrower, S., Hall, C., Lim, L. & Davison, A. N. (1976) *Biochem. J.* **160**, 271–280
- Thrower, S., White, J. O. & Lim, L. (1978) *Biochem. Soc. Trans.* **6**, 1312–1314
- Thrower, S., Neethling, C., White, J. O. & Lim, L. (1981) *Biochem. J.* **194**, 667–671
- Vertes, M. & King, R. J. B. (1971) *J. Endocrinol.* **5**, 271–282
- Vu Hai, M. T., Logeat, F. & Milgrom, E. (1978) *J. Endocrinology* **76**, 43–48
- Walters, M. R. & Clark, J. H. (1978) *Endocrinology* **103**, 601–609
- White, J. O. & Lim, L. (1980) *Biochem. J.* **190**, 833–837
- White, J. O., Neethling, C. & Lim, L. (1978a) *Abstr. Proc. Eur. Soc. Neurochem.* (Neuhoff, V. ed.), vol. 1, p. 622, Verlag-Chemie, Weinheim
- White, J. O., Thrower, S. & Lim, L. (1978b) *Biochem. J.* **172**, 37–47
- Young, P. C. M., Ehrlich, C. E. & Cleary, R. E. (1976) *Am. J. Obstet. Gynecol.* **125**, 353–360