Progesterone therapy results in partial reversibility of uterine abnormalities of the adult anovulatory rat

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The effects of progesterone therapy (5 mg, administered subcutaneously daily for 6 days) on the abnormal uterus of adult anovulatory Wistar rats have been studied. These rats, rendered anovulatory by neonatal treatment with testosterone propionate or clomiphene citrate, displayed severe hyperplasia and metaplasia of the uterine luminal epithelium and a disproportionately high content of nuclear oestrogen receptor, as a result of constant oestrogen stimulation unrelieved by progesterone [White, Moore, Elder & Lim (1981) *Biochem. J.* **196**, 557–565]. Progesterone therapy resulted in the virtual elimination of the hyperplasia and metaplasia and a corresponding decrease in the content of nuclear oestrogen receptor with the proportion of the unoccupied nuclear receptor being increased to values exhibited by normal cyclic females. There was also a decrease in the content of progestin receptors, a putative index of oestrogenic stimulation. Further, in the testosterone-treated group, progesterone therapy resulted in the restoration of oestrogen receptor translocational responses to oestradiol stimulation. Progesterone treatment of these anovulatory rats thus provides a model system for investigating the biochemical mechanisms underlying progestin antagonism and regulation of oestrogen-stimulated cell proliferation.

It is generally accepted that constant exposure to oestrogen unopposed by progesterone can lead to neoplasia in several tissues and organs (Clark & Peck, 1979). In the rat, administration of oestrogen or androgen during a critical neonatal period results in the adult female being anovulatory (Barracrough, 1967; Gorski, 1971). Such sterile anovulatory rats are deficient in progesterone (Sawada & Ichikawa, 1978) and are therefore exposed to relatively unopposed oestrogen concentrations in the range observed during normal dioestrus (Lobl & Maenza, 1975). This animal model therefore provides an opportunity to study the effects of unopposed oestrogen on the uterus and the clinically more relevant question of the association between endometrial hyperplasia and oestrogen therapy (Whitehead *et al.*, 1979).

We have previously reported (White *et al.*, 1981), in agreement with others (Lobl, 1975; Lobl & Maenza, 1975), that uterine morphology is abnormal in these adult anovulatory rats. There is an associated impairment of the normal oestrogenic response, as shown by a failure of oestradiol to promote oestrogen receptor translocation or to

induce the synthesis of progesterone receptors (White *et al.*, 1981). In the normal uterus many of the stimulatory effects of oestradiol are counteracted by progesterone (Martin, 1980); this has been the basis for the inclusion of progestins together with oestrogens during treatment of the climacteric (King & Whitehead, 1980). The anti-oestrogenic properties of progesterone are thought, in part, to be due to its participation in the down-regulation of the oestrogen receptor (Hsueh *et al.*, 1976; Coulson & Pavlik, 1977). The aim of the present study was to determine if the administration of progesterone to anovulatory rats would ameliorate some of the uterine effects, including the proliferation of the uterine epithelium, promoted by constant exposure to oestrogen. The results presented demonstrate that the uterine abnormalities induced by unopposed oestrogen are partially reversible. The changes in tissue morphology are accompanied by changes in oestrogen and progesterone receptor concentrations that are consistent with the anti-oestrogenic properties attributed to progesterone as well as by the partial restoration of normal oestrogen–receptor interactions.

Experimental

Production of adult anovulatory rats

Female Wistar rats were used throughout. Female pups (5 days old) were injected with either testosterone propionate (Sigma Chemical Co., Poole, Dorset, U.K.; 1.2 mg/animal) or clomiphene citrate ('Clomid'; a gift from Merrel, Slough, Berks., U.K.; 500 µg/animal), subcutaneously in oil as previously described (White *et al.*, 1981). At 120–140 days, animals showing a persistent oestrus-like vaginal histology were selected for experimentation.

Progesterone treatment of anovulatory rats

Progesterone (pregn-4-ene-3,20-dione, Sigma Chemical Co.) was dissolved in propylene glycol and administered subcutaneously in a constant volume (0.1 ml); each animal received 5 mg/day. Treatment was continued for 6 days. Animals were killed within 24–30 h of the last injection of progesterone.

Oestradiol administration

Animals were injected with 2.5 µg of oestradiol-17β [oestra-1,3,5(10)-triene-3,17-diol] (Sigma Chemical Co.) intraperitoneally in 0.2 ml of 50% (v/v) ethanol/saline 1 or 6 h before being killed. These injections were given to progesterone-treated rats 24 h after the final injection of progesterone.

Preparation of tissue and receptor determination

The preparation of uterine tissue for histological examination and the measurement of cytosol and nuclear receptors for oestrogen and progesterin was as previously described (White *et al.*, 1981). Briefly a longitudinal strip of one horn of the uterus was taken and fixed in phosphate-buffered 10% (v/v) saline before serial sectioning. The specific nuclear receptor was measured in triplicate 0.2 ml samples; for oestrogen a single saturating concentration of radioactive oestradiol ([2,4,6,7-³H]oestradiol-17β; 15 nM; sp. radioactivity 87–99 Ci/mmol; The Radiochemical Centre, Amersham, Bucks., U.K.) was used with and without a 200-fold excess of diethylstilboestrol. Incubations were for 1 h at 30°C (to measure total receptor) and at 4°C (to measure unoccupied receptor) (White *et al.*, 1981). Nuclear

progesterin receptors were measured by using a single saturating concentration of progesterone ([1,2,6,7-³H]progesterone; 20 mM; sp. radioactivity 90–110 Ci/mmol; The Radiochemical Centre) at 4°C for 18–24 h. Unlabelled progesterone (100-fold excess) was used as competitor; cortisol (100-fold excess) was included to minimize the binding of radioactive progesterone to corticosteroid-binding globulin. The specific cytosol receptor was measured in duplicate 0.2 ml samples at 4°C for 18–24 h; for oestrogen receptor 5 nM-[³H]oestradiol in the absence or presence of a 200-fold excess of diethylstilboestrol was used. Cytosol progesterin receptor was measured using the ligand concentration described for the nuclear receptor. Macromolecular bound radioactivity in the cytosol fractions was separated from unbound steroid by chromatography on columns of Sephadex LH-20 (Thrower *et al.*, 1976).

DNA and protein estimations

These were determined by the procedures of Burton (1956) and Lowry *et al.* (1951) respectively.

Monitoring of radioactivity

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

Results

Histology of uteri after progesterone therapy

The uteri of clomiphene- and testosterone-treated anovulatory rats (White *et al.*, 1981) characteristically displayed an increase in activity of the cells of the luminal epithelium, which were predominantly columnar and hyperplastic. Pronounced but scattered areas of metaplasia were observed, which often appeared to be gland-associated. The glands were decreased in size and number, and in some there was evidence of metaplasia. The endometrium was thin and, in some cases, eroded; there was no evidence of malignancy.

Progesterone therapy resulted in changes of the uterine wall (Plate 1); the most noticeable changes

EXPLANATION OF PLATE 1

Histology of uteri

(a) Longitudinal section (magnification × 25) of uteri from anovulatory adult female rats. (i) Testosterone propionate group (no progesterone therapy). Arrows indicate areas of epithelial (a) hyperplasia and (b) metaplasia. (ii) Testosterone propionate group after 6 days of progesterone therapy. Arrows indicate stromal glands, now present in increased numbers. (iii) Clomiphene citrate group (no progesterone therapy). Arrow indicates area of metaplasia. (iv) Clomiphene citrate group after 6 days of progesterone treatment. Arrows indicate stromal glands, now present in increased numbers. (b) Longitudinal section (magnification × 75) of uteri from anovulatory adult female rats to show changes in luminal epithelium. Designation of (i)–(iv) is as in (a). This more highly magnified series shows clearly areas of epithelial metaplasia and hyperplasia in (i) and (iii), which virtually disappear on progesterone therapy in (ii) and (iv).

were in the luminal epithelium, which in both groups was predominantly cuboidal with a decrease in the amount of hyperplasia and metaplasia. There was an increase in the number and size of the glands, with associated areas of metaplasia. The endometrial layer was enlarged and more densely stained than in the groups not subjected to progesterone therapy.

Effects of progesterone therapy on the uterine oestrogen-receptor content and its response to oestradiol administration

Testosterone-treated group. Progesterone therapy did not affect the content of cytosol oestrogen receptor (Table 1a). The administration of oestradiol 1 h before death resulted in a depletion of receptor content, regardless of prior treatment with progesterone. Partial replenishment of receptor was observed 6 h after oestradiol administration. The content of nuclear oestrogen receptor was decreased

after progesterone therapy (Table 1a). At 1 h after oestradiol administration to animals subjected to progesterone therapy there was an increase in nuclear oestrogen receptor content that paralleled the depletion of cytosol receptor. The apparent increase in nuclear receptor content was greater than the observed depletion in cytosol receptor content; however, there was no significant difference in the total cellular content of receptor. In animals that had not been subjected to progesterone therapy the oestrogen-stimulated depletion of cytosol receptor was not accompanied by an increase in nuclear receptor content. The content of nuclear receptor remained elevated 6 h after oestradiol administration in the rats subjected to therapy.

Clomiphene-treated group. Progesterone therapy resulted in a decrease in the content of cytosol oestrogen receptor (Table 1b); oestradiol administration did not deplete this already low receptor

Table 1. *The effect of progesterone therapy on the content of cytosol and nuclear oestrogen receptors of neonatally treated anovulatory rats and their response to oestradiol administration*

Adult anovulatory rats in persistent vaginal oestrus were selected by examination of vaginal smears; each animal was used as a single experiment. On six consecutive days each animal was injected subcutaneously with 5 mg of progesterone (in 0.1 ml of propylene glycol); on day 7 uterine receptor content was measured either before or 1 and 6 h after the administration of 2.5 µg of oestradiol intraperitoneally (0.2 ml of ethanol/0.9% saline, 1:1, v/v). Tissue preparation, fractionation and measurement of receptor were as described in the Experimental section; each value is the mean ± s.d. of five estimations using rats treated neonatally with either testosterone propionate or clomiphene citrate. Data from animals not treated with progesterone (four separate estimations; three animals/estimation) are presented for comparison. Statistical analysis was by two-tailed Student's *t* test. *, Significantly different from 'no progesterone' ($P < 0.05$); **, significantly different from 'no progesterone' ($P < 0.02$); ***, significantly different from 'no progesterone' ($P < 0.005$); †, significantly different from '0 h' ($P < 0.001$). ††, significantly different from '0 h' ($P < 0.01$); †††, significantly different from '0 h' ($P < 0.005$).

(a) Testosterone-treated group		No progesterone therapy			After progesterone therapy		
Time after 2.5 µg of oestradiol treatment (h)	...	0	1	6	0	1	6
Oestrogen receptor content (pmol/g wet wt.)							
Nuclear (30°C)		3.73	3.88	3.06	1.98*	7.56†	4.30
		±0.95	±1.37	±2.30	±0.96	±1.52	±0.82
Cytosol		3.17	0.32	1.58	2.44	0.60	1.71
		±1.42	±0.25	±1.00	±0.69	±0.49	±0.42
Nuclear + cytosol		6.90	4.27	4.64	5.41	7.65	5.76
		±0.92	±1.27	±2.07	±2.4	±1.59	±0.81
Tissue wet wt. (g)		0.36	0.33	0.37	0.39	0.44	0.51††
		±0.04	±0.04	±0.07	±0.06	±0.05	±0.05
(b) Clomid-treated group		No progesterone therapy			After progesterone therapy		
Time after 2.5 µg of oestradiol treatment (h)	...	0	1	6	0	1	6
Oestrogen receptor content (pmol/g wet wt.)							
Nuclear (30°C)		11.35	11.87	2.19	6.53**	5.90	3.93
		±2.75	±5.10	±0.37	±1.25	±1.17	±1.29
Cytosol		4.95	1.21	1.47	1.34***	1.99	4.00
		±1.31	±0.66	±0.17	±0.67	±1.55	±1.89
Nuclear + cytosol		16.30	13.15	3.66	7.87	7.89	7.93
		±1.48	±5.05	±0.45	±1.52	±1.96	±1.88
Tissue wet wt. (g)		0.22	0.17	0.23	0.23	0.27	0.39†††
		±0.07	±0.04	±0.02	±0.05	±0.07	±0.06

Table 2. *Unoccupied nuclear oestrogen receptors: changes after progesterone therapy*

Animals were selected and treated as described in the legend to Table 1. Unoccupied nuclear receptors were measured at 4°C for 1 h. Data are means $\pm$ s.d. of five estimations; data from animals not treated with progesterone are shown for comparison. The relationship between unoccupied and total receptors is expressed as the ratio: $100 \times (\text{nuclear receptor at } 4^\circ\text{C})/(\text{nuclear receptor at } 30^\circ\text{C})$. Statistical analysis was by Student's *t* test: *, significantly different from 'no progesterone' ($P < 0.05$); **, significantly different from 'no progesterone' ($P < 0.025$); ***, significantly different from 'no progesterone' ($P < 0.0025$); †, significantly different from 'no progesterone' ($P < 0.0005$).

		No progesterone therapy			After progesterone therapy		
Time after 2.5 μ g of oestradiol treatment (h) ...		0	1	6	0	1	6
Nuclear receptor at 4°C (pmol/g of tissue)		2.10	0.84	1.08	1.78	6.09	3.43
		± 0.21	± 0.26	± 0.06	± 0.42	± 1.68	± 0.41
100 $\times$ (Nuclear receptor at 4°C)/(nuclear receptor at 30°C)		49.80	25.20	42.40	80.10*	93.70†	89.00**
		± 3.75	± 11.90	± 22.90	± 34.50	± 23.70	± 30.00
(b) Clomiphene-treated group		No progesterone therapy			After progesterone therapy		
Time after 2.5 μ g of oestradiol treatment ...		0	1	6	0	1	6
Nuclear receptor at 4°C (pmol/g of tissue)		4.83	3.47	1.85	6.31	3.08	3.35
		± 3.15	± 2.44	± 0.24	± 1.68	± 0.66	± 0.72
100 $\times$ (Nuclear receptor at 4°C)/(nuclear receptor at 30°C)		39.70	29.50	86.10	111.60***	55.30*	91.80
		± 16.40	± 17.80	± 5.60	± 31.00	± 26.00	± 26.90

Table 3. *Progesterin receptor concentrations after progesterone therapy*

Animals were selected and treated as described in the legend to Table 1. Oestradiol administration had no effect on progesterone receptor concentrations; the data are therefore expressed as means $\pm$ s.d., regardless of injection with oestradiol. The numbers in parentheses represent the total number of determinations. Statistical analysis was by Student's *t* test. *, Significantly different from 'no progesterone' ($P < 0.0025$); **, significantly different from 'no progesterone' ($P < 0.005$).

		Progesterin receptor content	
		No progesterone therapy	After progesterone therapy
(a) Testosterone-treated group			
Cytosol (fmol/mg of protein)		116.2 $\pm$ 67.0 (11)	44.33 $\pm$ 25.46* (15)
Nuclear (pmol/mg of DNA)		1.17 $\pm$ 0.32 (12)	0.71 $\pm$ 0.36 (15)
(b) Clomiphene-treated group			
Cytosol (fmol/mg of protein)		118.9 $\pm$ 77.2 (10)	46.89 $\pm$ 18.95* (10)
Nuclear (pmol/mg of DNA)		2.35 $\pm$ 1.27 (12)	0.73 $\pm$ 0.36 (15)

content. In the group not subjected to progesterone therapy oestradiol administration caused a depletion of cytosol oestrogen receptor. As in the testosterone-treated group, progesterone therapy resulted in a significant decrease in nuclear oestrogen receptor content (Table 1b). However, oestradiol administration in this case did not result in an increase in the content of nuclear oestrogen receptors.

Unoccupied nuclear receptors

Unoccupied nuclear receptors were present in both the testosterone- and clomiphene-treated groups (Table 2). In the testosterone-treated group, after progesterone therapy the majority of the nuclear oestrogen receptors were unoccupied at all times after oestradiol administration. These unoccupied receptors represented a greater proportion of

the total nuclear receptor content when compared with the corresponding group not subjected to progesterone therapy. The observation that the majority of nuclear receptors, in normal tissue, are unoccupied has previously been reported by our laboratories (White & Lim, 1980; Thrower & Lim, 1980; Thrower *et al.*, 1981). The majority of nuclear receptors in the clomiphene-treated group were also unoccupied after progesterone therapy.

Progesterin receptor

Both the cytosol and nuclear progesterin receptor content, measured 24 h after the final administration of progesterone, were decreased in the testosterone- and clomiphene-treated groups after progesterone therapy (Tables 3a and 3b). These observations are consistent with progesterone inhibition of the synthesis of progesterin receptor (Milgrom *et al.*, 1973).

Discussion

In testosterone and clomiphene-treated anovulatory animals the increased and abnormal proliferation of the luminal epithelial cell layer may be indicative of a pre-malignant condition resulting from constant exposure to oestrogen, unopposed by progesterone (White *et al.*, 1981). Progesterone therapy resulted in a lowered incidence of hyperplasia and metaplasia in both groups, probably as a result of the anti-oestrogenic action of progesterone. There was also evidence of a proliferative effect of progesterone as reflected by an increase in the number of glands observed in the uterine stroma of both groups (Plate 1); this, together with the low cuboidal luminal epithelium, is characteristic of a tissue under progestin influence (Hsueh *et al.*, 1979). Histological evidence therefore suggests that it is possible to arrest the abnormal proliferation (induced by oestrogen) of these uteri and restore normal morphology by progesterone therapy. This restoration is associated with a partial recovery of normal oestrogen-receptor interactions (Tables 1 and 2).

In normal adult female rats, regulated changes in the concentrations of oestradiol and progesterone lead to cyclic changes in uterine morphology (Brenner & West, 1975). It is generally accepted that progesterone, peak concentrations of which follow those of oestrogens in the cycle, serves to antagonize oestrogen-promoted epithelial proliferation and to induce stromal proliferation (Finn & Martin, 1974). Changes in oestrogen and progesterone receptor distribution within the cell parallel these hormonal changes (White *et al.*, 1978; Vu Hai *et al.*, 1978; Myatt *et al.*, 1978) consistent with their involvement in the hormonal response. If, as in other tissues (Buller & O'Malley, 1976), the nuclear oestrogen receptor is assumed to be biologically active, then the decrease in nuclear oestrogen receptor content in both groups of neonatally treated rats after progesterone therapy suggests a progesterone-induced limitation of the oestrogenic stimulation responsible for the abnormal morphology. From the results presented the neonatally testosterone-treated rats appear to serve as a better model for studying progesterone antagonism of oestrogenic action. The differences between the two groups of neonatally altered rats may lie in the differential neural and peripheral effects of testosterone and clomiphene during the critical neonatal period (Aihara *et al.*, 1980).

In the clomiphene-treated group subjected to progesterone therapy the decrease in nuclear receptor content appeared to be the result of a decreased availability of cytosol oestrogen receptor for translocation (Table 1). Such effects of progesterone are consistent with the proposals of Coulson & Pavlik (1977). In contrast, progesterone therapy had a

different effect on the testosterone-treated group; cytosol oestrogen receptor remained unaltered despite a decrease in nuclear oestrogen receptor content. Changes in nuclear but not cytosol uterine oestrogen receptor content are also observed during the oestrous cycle and in early pregnancy (White *et al.*, 1978; Myatt *et al.*, 1978, 1980a). There is evidence that factors other than cytosol receptor content may determine the extent of hormone action; these include nuclear acceptor sites (Buller & O'Malley, 1976) and substances that either increase (Thrower *et al.*, 1976) or inhibit activation (Sato *et al.*, 1978; Shen *et al.*, 1979) of the oestrogen receptor obligatory for nuclear binding. Progesterone antagonism of oestrogen stimulation may in part be mediated via the modulation of the concentration of such factors. Progestin receptor synthesis in the rodent uterus is itself regulated by oestrogen (Feil *et al.*, 1972) and changes in nuclear oestrogen receptor levels correlate with changes in progestin receptor synthesis (Thrower & Lim, 1980; Myatt *et al.*, 1980b). Despite the occurrence of substantial concentrations of nuclear oestrogen receptors the content of progestin receptor in the neonatally treated anovulatory rats was found to be lower than in normal animals (White *et al.*, 1981) suggesting a defect in the regulation of progestin receptor synthesis. Nevertheless, despite this defective oestrogen-mediated synthesis, progesterone administration still potentiated a decrease in total progestin receptor content in both groups of neonatally altered rats (Table 3); this is consistent with an inhibitory effect of progesterone on the synthesis of progestin receptors (Leavitt *et al.*, 1978) and with our other evidence that the abnormal uterus is responsive to progesterone.

In both the testosterone- and clomiphene-treated groups there was an impairment of the receptor response to exogenous oestradiol (White *et al.*, 1981). After progesterone therapy the content of cytosol oestrogen receptor in the clomiphene-treated group decreased (Table 1). The residual receptor was not further depleted by oestradiol, suggesting a complete insensitivity to oestrogen after progesterone therapy. In contrast, after progesterone therapy the testosterone-treated group responded to exogenous oestradiol by a depletion of cytosol receptor, which was accompanied by an increase in nuclear receptor content. However, the increment in nuclear receptor apparently exceeded the decrease in cytosol receptor (Table 1a). Although no significant difference in the total content (nuclear plus cytosol) of receptor was found after oestradiol administration, it is possible that this discrepancy reflects some persistent defect in the processing of nuclear oestrogen receptors (Horwitz & McGuire, 1978; Clark & Peck, 1979), even after progesterone therapy. In the progesterone-treated rat,

the content of nuclear oestrogen receptor still remained high 6h after oestradiol administration (Table 1), which contrasts with the response of normal adult rats (Thrower & Lim, 1981) and which supports the suggestion that nuclear processing is somewhat impaired.

The histological changes after progesterone therapy of both groups were also associated with changes in the unoccupied component of the nuclear oestrogen receptor. Unoccupied receptors were first reported in mammary tumours (Zava *et al.*, 1977) and subsequently in normal tissue (White & Lim, 1980; Thrower & Lim, 1980; Levy *et al.*, 1980; King *et al.*, 1980). However, both cytosolic contamination (Edwards *et al.*, 1980) and preparative artefacts (Martin & Sheridan, 1980) have been reported to be responsible for the presence of unoccupied receptors in the breast-tumour cell line MCF-7. Nevertheless, there is strong evidence from our own (Thrower *et al.*, 1981) and other (Levy *et al.*, 1980; King *et al.*, 1980) laboratories that in normal tissue the uterine unoccupied nuclear receptor is not the result of cytosolic contamination. Moreover, in the normal rat uterus the major proportion of nuclear receptors is unoccupied and remains so throughout the oestrous cycle (Thrower & Lim, 1980). In the anovulatory rats the proportion of nuclear receptors that was unoccupied was consistently raised after progesterone therapy (Table 2). The occurrence of unoccupied receptors in lower than normal proportions may thus be indicative of some abnormality in the tissue. We have also previously presented evidence that in the abnormal hypothalamus of the neonatally androgenized rat the proportion of unoccupied receptors is lower than in normal animals (White & Lim, 1980).

Our results therefore suggest that the neonatally testosterone-treated anovulatory rat represents a good model for studying the effects of progesterone therapy on uteri exposed constantly and maximally (because unopposed) to oestrogenic stimulation. Thus the morphological abnormalities appear to be related to the extremely high levels of nuclear oestrogen receptors, which also display abnormal properties. The antagonism by progesterone of the proliferative effect of oestrogen is seen to be associated with partial restoration of the normal oestrogenic receptor interactions, including a decrease in the abnormally high content of nuclear oestrogen receptors and the ability to translocate oestrogen receptors on oestradiol stimulation. Whether progesterone withdrawal re-initiates morphological and biochemical abnormalities is an interesting question. These studies should be viewed in the context of current hormone regimens used in the treatment of the climacteric.

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References

- Aihara, M., Takeji, K. & Kato, J. (1980) *Endocrinology* **107**, 224–230
- Barracough, C. A. (1967) *Neuroendocrinology* **2**, 62–99
- Brenner, R. M. & West, N. B. (1975) *Annu. Rev. Physiol.* **37**, 273–302
- Buller, R. E. & O'Malley, B. W. (1976) *Biochem. Pharmacol.* **25**, 1–12
- Burton, K. (1956) *Biochem. J.* **62**, 315–323
- Clark, J. H. & Peck, E. J. Jr. (1979) *Female Sex Steroids: Receptors and Function*, p. 135, Springer-Verlag, Berlin, Heidelberg and New York
- Coulson, P. B. & Pavlik, E. J. (1977) *J. Steroid Biochem.* **8**, 205–212
- Edwards, D. P., Martin, P. M., Horwitz, K. B., Chamness, G. C. & McGuire, W. L. (1980) *Exp. Cell Res.* **127**, 197–213
- Feil, P. D., Glasser, S. R., Toft, D. O. & O'Malley, B. W. (1972) *Endocrinology* **91**, 738–746
- 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
- Horwitz, K. B. & McGuire, W. L. (1978) *J. Biol. Chem.* **253**, 2223–2228
- Hsueh, A. J. W., Peck, E. J. Jr. & Clark, J. H. (1976) *Endocrinology* **98**, 438–444
- Hsueh, A. J. W., Erickson, G. F. & Lu, K. H. (1979) *Biol. Reprod.* **21**, 793–800
- King, R. J. B. & Whitehead, M. I. (1980) in *Perspectives in Steroid Receptor Research* (Bresciani, F., ed.), pp. 259–271, Raven Press, New York
- King, R. J. B., Dyer, G., Collins, W. P. & Whitehead, M. I. (1980) *J. Steroid Biochem.* **13**, 377–382
- 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, New York, Academic Press
- 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
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
- Martin, L. (1980) in *Estrogens in the Environment* (McLachlan, G., ed.), pp. 103–130, Elsevier/North-Holland, Amsterdam
- Martin, P. M. & Sheridan, P. J. (1980) *Experientia* **36**, 620–622
- Milgrom, E., Thi, L., Atger, M. & Baulieu, E.-E. (1973) *J. Biol. Chem.* **248**, 6366–6377
- Myatt, L., Chaudhuri, G., Elder, M. G. & Lim, L. (1978) *Biochem. J.* **176**, 523–529
- Myatt, L., Chaudhuri, G., Elder, M. G. & Lim, L. (1980a) *J. Endocrinol.* **87**, 357–364
- Myatt, L., Chaudhuri, G., Elder, M. G. & Lim, L. (1980b) *J. Endocrinol.* **87**, 365–373
- Sato, B., Huseby, R. A. & Samuels, L. T. (1978) *Endocrinology* **102**, 545–555
- Sawada, T. & Ichikawa, S. (1978) *Endocrinology* **102**, 1436–1444

- Shen, G., Thrower, S. & Lim, L. (1979) *Biochem. J.* **182**, 241–243
- Thrower, S. & Lim, L. (1980) *Biochem. J.* **190**, 691–695
- Thrower, S. & Lim, L. (1981) *Biochem. J.* **198**, 385–389
- Thrower, S., Hall, C., Lim, L. & Davison, A. N. (1976) *Biochem. J.* **160**, 271–280
- Thrower, S., Neethling, C., White, J. O. & Lim, L. (1981) *Biochem. J.* **194**, 667–671
- Vu Hai, M. T., Logeat, F. & Milgrom, E. (1978) *J. Endocrinol.* **76**, 43–48
- White, J. O. & Lim, L. (1980) *Biochem. J.* **190**, 833–837
- White, J. O., Thrower, S. & Lim, L. (1978) *Biochem. J.* **172**, 37–47
- White, J. O., Moore, P. A., Elder, M. G. & Lim, L. (1981) *Biochem. J.* **196**, 557–565
- Whitehead, M. I., King, R. J. B., McQueen, J. & Campbell, S. (1979) *J. R. Soc. Med.* **72**, 322–327
- Zava, D. T., Chamness, G. C., Horwitz, K. B. & McGuire, W. L. (1977) *Science* **196**, 663–664