The Oestrogen Receptor in the Rat Uterus in Relation to Intra-Uterine Devices and the Oestrous Cycle

By LESLIE MYATT,*† GAUTUM CHAUDHURI,* MURDOCH G. ELDER*†
and LOUIS LIM‡

*Department of Obstetrics and Gynaecology, Charing Cross Hospital Medical School,
West London Hospital, Hammersmith Road, London W6 8RP, U.K., and

†Department of Neurochemistry, Institute of Neurology, The National Hospital,
Queen Square, London WC1N 3BG, U.K.

(Received 21 April 1978)

We investigated the binding characteristics, content and intracellular distribution of nuclear and cytosolic oestrogen receptors in the uteri of rats bearing a unilateral intra-uterine device, fitted 14–18 days earlier, at four phases of a 5-day oestrous cycle. The patterns of changes in wet weight and content of cytosolic and nuclear receptor that normally occur during the oestrous cycle were not altered by the presence of the device. At all stages of the cycle the intra-uterine-device-containing horn had a greater wet weight and a correspondingly higher content of cytosolic receptor than its contralateral control horn, the cellular concentration of cytosolic receptor being apparently maintained. However, the intra-uterine-device-containing horn had significantly lower cellular concentrations (i.e. per mg of DNA) of nuclear receptor, particularly at late dioestrus and pro-oestrus. Thus the treated horn showed a decreased translocation of receptor in response to increases in circulating oestrogens. Both horns contained equivalent amounts of an activating factor implicated in translocation and measured *in vitro* by binding of cytosol receptor to oligo(dT)–cellulose. The presence of an intra-uterine device neither altered the dissociation constants (K_d) of the nuclear and cytosolic oestrogen–receptor complexes nor the stability of the nuclear receptor complex *in vitro*. The decreased translocation cannot thus be directly attributed to changes in the physical properties of the receptor. This decrease may be responsible for the anti-fertility effect of the intra-uterine device (which affects only the treated horn of the bicornuate rat uterus), since implantation of the blastocyst requires correct concentrations of nuclear oestrogen receptor.

The role of a specific macromolecular receptor present in the cytosol of target tissues in oestrogen-mediated cell functions is now well established (O'Malley & Means, 1974; King & Mainwaring, 1974). This cytosolic receptor forms a complex with oestrogen entering the cell and then translocates into the nucleus, where it influences cellular activity. In the rat uterus, the extent of the cellular response appears to be governed by the concentration of the nuclear receptors, and cellular changes that occur during the oestrous cycle are associated with phasic changes in nuclear receptor content (Clark *et al.*, 1972, 1973; White *et al.*, 1978).

The reproductive function of the rat uterus can be interfered with by the insertion of an intra-uterine device. However, the anti-fertility effect is seen only in the intra-uterine-device-containing horn, the

other horn of the bicornuate uterus supporting normal implantation of the blastocyst (Doyle & Margolis, 1964). In other species, for example the mouse, both horns can be affected. In the rat, this insertion results in a generalized growth of both endometrium and myometrium, accompanied by increases in wet and dry weight. The presence of the intra-uterine device does not alter the duration of the oestrous cycle, ovulation, gonadotropin release or corpus-luteum function (Parr & Segal, 1966; Corfman & Segal, 1968). The metabolic effects observed include increased incorporation of precursors into RNA and protein and have been attributed to possible alterations in the sensitivity of the affected uterus to circulating oestrogens, as a result of increased intracellular concentrations of either oestrogen or oestrogen receptors (Laumas & Yadava, 1969; Joshi, 1970). Labhsetwar & Perser (1972) have also reported an increased uptake of radioactive oestradiol throughout the oestrous cycle by rat uteri containing intra-uterine devices. However, the study

† Present address: Department of Obstetrics and Gynaecology, Institute of Obstetrics and Gynaecology, Hammersmith Hospital, Ducane Road, London W120HS, U.K.

involved the injection of trace amounts of [^3H]-oestradiol into normal adults, where endogenous concentrations of circulating oestrogens undoubtedly influenced measurements of apparent uptake of the hormone.

Oestradiol is involved in the implantation process (Heald, 1976), and the participation of nuclear oestrogen receptors is critical to this involvement (Martel & Psychoyos, 1976). Because of the pivotal role of oestrogens in uterine functions we have investigated the effects of the presence of an intra-uterine device in uterine horns on oestrogen-binding characteristics of the oestrogen receptor as well as on its distribution in the cytosol and nuclei throughout the oestrous cycle, with the contralateral untreated horns as controls.

Experimental

Materials

[2,4,6,7(n)- ^3H]Oestradiol-17 β (sp. radioactivity 100Ci/mmol) was supplied by The Radiochemical Centre, Amersham, Bucks., U.K. Oligo(dT)-cellulose was obtained from Collaborative Research, Waltham, MA, U.S.A., supplied by Uniscience, Cambridge, U.K. Sephadex LH-20 was from Pharmacia (G.B.), London W.5, U.K. Bio-Solv BBS 3 was from Beckman RIIC, Hitchin, Herts., U.K. Butyl-PBD [5-(biphenyl-4-yl)-2-(4-t-butylphenyl)-1-oxa-3,4-diazole] was from Fisons, Loughborough, Leics., U.K. Silk sutures, 2/0 gauge, were supplied by Ethicon, Edinburgh, Scotland, U.K. Female Wistar rats were bred in the Animal Unit, Charing Cross Hospital Medical School.

Fitting of intra-uterine devices and monitoring of oestrous cycle

Groups of 30 female rats aged 70 ± 1 days were fitted with a silk suture through the entire lumen of one uterine horn, under fluothane anaesthesia. A sham operation was carried out on the contralateral uterine horn. After a period of 14 days, vaginal smears were taken for several successive days to monitor the progress of the oestrous cycle; on day 4, rats were grouped according to the stage of the oestrous cycle. Some 95% of the rats followed a 5-day oestrous cycle, with the dioestrus phase lasting 3 days. The stages that we studied were pro-oestrus (day 1), oestrus (day 2), metoestrus/early dioestrus (day 3) and late dioestrus (day 5).

Preparation of uterine material

Animals were killed by decapitation at 09:00h on the day of the experiment. The uterine horns were excised, stripped of fat and placed in ice-cold TED

buffer [10mM-Tris/HCl (pH 7.6)/1.5mM-EDTA/1mM-dithiothreitol]. All subsequent procedures were carried out at 4°C. Excess of buffer was removed by placing the uteri on filter paper before they were weighed, finely chopped with scissors and homogenized in 6vol. of TED buffer by using a type X10/20 homogenizer (The Scientific Instrument Centre, London W.C.1, U.K.), in two bursts of 10s with constant cooling. The homogenate was centrifuged at 1200g for 10min at 4°C. This yielded a crude nuclear-myofibrillar pellet. The supernatant was further centrifuged at 200000g for 90min in the MSE 8 $\times$ 25 A1 rotor at 4°C to give the cytosol fraction. The nuclear pellet was washed four times in the original volume of buffer, before resuspension by using a vortex mixer and gentle aspiration through a Pasteur pipette.

Assay of nuclear oestrogen receptors

For the characterization of oestrogen binding to the nuclear receptor, triplicate samples of the nuclear suspension (0.2ml) were incubated at 37°C for 1h with different concentrations of [^3H]oestradiol (0.25–10nM) with or without a 200-fold excess of diethylstilboestrol (Anderson *et al.*, 1972). Under these conditions endogenous oestrogen was exchanged with the added labelled oestradiol and available binding sites were filled by [^3H]oestradiol. For routine measurements of total nuclear receptors, a saturating concentration of 10nM-[^3H]oestradiol was used. The exchange was terminated after 1h by the addition of 1ml of ice-cold TED buffer. The nuclear pellet was then washed three times with TED buffer. To decrease the high non-specific binding (from 50–70% to 30% of total radioactivity) an additional 5min wash with 1ml of 0.1% Triton X-100 in TED buffer was introduced at the beginning of the washes. [^3H]Oestradiol was then extracted with 2 $\times$ 1ml of ethanol. The extracts were added to 10ml of a scintillation 'cocktail' of 4.5g of butyl-PBD in 1 litre of toluene for determination of radioactivity in an Intertechnique SL 4000 liquid-scintillation counter. Triplicate estimations agreed to within 10–15%. The rate of receptor breakdown was 15%/hour for both treated and control horns and estimates of nuclear receptor were accordingly adjusted.

Assay of cytosolic receptor

For analysis of cytosolic receptor binding of oestradiol, duplicate samples (0.2ml) of cytosol were incubated overnight at 4°C with various concentrations of [^3H]oestradiol (0.25–10nM) with or without a 200-fold excess of diethylstilboestrol. For routine measurements of cytosolic receptors a saturating concentration of 5nM-[^3H]oestradiol was used. The cytosolic samples were then chromato-

graphed on columns (6cm×0.6cm) of Sephadex LH-20 (Ginsburg *et al.*, 1974; Thrower *et al.*, 1976). The [³H]oestradiol-receptor complex was eluted in the void volume, whereas free [³H]oestradiol was retarded in the gel. The void volume (1.0ml) was collected and added to 10ml of the previously described scintillation 'cocktail', containing in addition 10% Bio-Solv BBS 3, for determination of radioactivity. Duplicate measurements agreed to within 5% and non-specific binding was routinely less than 10% of total bound radioactivity. This method measures the majority of cytosolic receptors since the effect of endogenous oestradiol has been shown to be minimal (see Thrower *et al.*, 1976; White *et al.*, 1978).

Binding of cytosol receptor to oligo(dT)-cellulose

Duplicate samples (0.2ml) of cytosol, incubated first with 5 nM-[³H]oestradiol with or without excess of diethylstilboestrol for 18 h at 4°C, were then incubated with 100 mg of oligo(dT)-cellulose for 1 h at 4°C (Thrower *et al.*, 1976). Free [³H]oestradiol and oestrogen-receptor complexes not bound to the oligo(dT)-cellulose matrix were eluted with 15 ml of TED buffer. Bound complexes were eluted with 0.6 M-KCl/TED buffer. Final washes with TED buffer (the last 1.0 ml sample) and 0.6 M-KCl/TED buffer (4×1.0 ml) were collected separately for measurement of radioactivity as described previously. Duplicate measurements agreed to within 2–5%; results were expressed as a percentage of cytosolic oestrogen receptor content.

Assay of DNA

DNA was measured by the method of Burton (1956).

Results

Interaction of oestrogen with receptors isolated from intra-uterine-device-containing and control horns

We first set out to determine whether the presence of an intra-uterine device might grossly affect the interaction of oestrogen with the receptor. In addition, it was necessary to validate the use of methods previously used (White *et al.*, 1978) in determining the content of oestrogen receptors in nuclear and cytosolic fractions isolated from uteri of normal females. The exchange assay method of Anderson *et al.* (1972) was used with a slight modification (see the Experimental section). In both intra-uterine-device-containing and control horns, the maximum complexing of nuclear oestrogen receptor with [³H]oestradiol occurred at a concentration of

10 nM-[³H]oestradiol. This concentration of [³H]oestradiol was used in all subsequent measurements of nuclear receptor. Analysis of the binding data, by the method of Scatchard (1949), showed the nuclear receptor isolated from intra-uterine-device-containing horns and from control horns to have dissociation constants (K_d) ($\pm$ s.e.m.) of respectively 4.1 ± 0.3 (4) and 2.2 ± 0.7 (4) nM. These are in the range of K_d values for nuclear receptors reported by other workers (see Buller & O'Malley, 1976) and showed that the presence of an intra-uterine device had not substantially altered the binding characteristics of the nuclear receptor.

In both intra-uterine-device-containing and control horns, 95% saturation of the specific binding sites on the cytosol receptors was obtained at a concentration of 5 nM-[³H]oestradiol. This concentration was used in all subsequent measurements of the cytosolic receptor. Scatchard analysis of the binding data gave a K_d of 0.40 ± 0.03 (3) and 0.46 ± 0.04 (3) nM for cytosolic receptors isolated from intra-uterine-device-containing and control horns respectively. These measurements, which are in agreement with other reports (Buller & O'Malley, 1976), showed the intra-uterine device to have no effect on the oestrogen-binding characteristics of the cytosolic receptor.

Effect of the intra-uterine device on the oestrous cycle

(a) *Phasic changes in wet weight and oestrogen-receptor content.* We next studied the effects of the intra-uterine device on the phasic changes in wet weight and in receptor content normally associated with the oestrous cycle. The data were derived from measurements of four separate groups of rats, with each group being segregated into the different phases of the cycle. The results for each phase were expressed in terms of the mean value of all four separate phases (mean = 100%). The results for the different groups of animals could then be combined to give mean values $\pm$ s.e.m. This kind of analysis is similar to that of White *et al.* (1978). The results (Fig. 1) showed that both intra-uterine-device-containing and control horns exhibited similar patterns of changes in wet weight and content of cytosol and nuclear receptors. For example, in both horns, wet weight and nuclear receptor content were minimal at met-oestrus and maximal at pro-oestrus. The intra-uterine device thus did not alter the basic phasic nature of uterine activity. However, it appeared to affect the magnitude of the phasic changes in nuclear receptor content. In the transition from metoestrus to pro-oestrus, the control uterine horn responded to the increase in circulating oestrogen (Naftolin *et al.*, 1972) with a corresponding increase in nuclear receptor content of about 5-fold (similar to that reported by White *et al.*, 1978). In the intra-uterine-

Fig. 1. Comparison of phasic changes during the oestrous cycle in wet weight, cytosolic receptor content and nuclear receptor content for intra-uterine-device-containing and control uterine horns

Four separate groups of 30 rats, fitted unilaterally with an intra-uterine device 18 days previously, were segregated according to phase of oestrous cycle. Measurements of uterine wet weight, cytosolic and nuclear receptor content were made as described (and tabulated) in Tables 1 and 2 and in the Experimental section. The mean value for each parameter over the four different phases for each group was calculated (means = 100%). Values of the individual phases (mean $\pm$ S.E.M.) are expressed in relation to the group mean. Symbols: □, wet weight; ■, cytosolic receptor content; ▨, nuclear receptor content. (a) Control uterine horns; (b) intra-uterine-device-containing uterine horns. Phases of the oestrous cycle measured were: P, pro-oestrus; O, oestrus; M, metoestrus/early dioestrus; D, late dioestrus.

device-containing horn the equivalent increase was only about 3-fold (cf. Figs. 1a and 1b; metoestrus against pro-oestrus).

(b) *Intra-uterine-device-induced changes in wet weight and cytosolic receptor content.* At all four phases of the oestrous cycle, the presence of the intra-uterine device increased the wet weight of the affected horn (Table 1A). This increase varied from 30 to 60%, being greater at late dioestrus and pro-oestrus. The increase was accompanied by corresponding increases in cytosolic receptor content (Table 1B). In both horns, cytosolic receptor concentration was maximal at the late dioestrus phase.

The capacity of the cytosolic oestrogen receptor to bind to oligo(dT)cellulose was also determined. This binding capacity *in vitro* has been suggested to reflect possibly the potential for translocation *in vivo* and long-term nuclear binding and is low in tissues with limited nuclear binding of receptor (Thrower *et al.*, 1976; White *et al.*, 1978). Although the presence of an intra-uterine device in the horn appeared generally to decrease the binding capacity at all phases, with lowest values being at pro-oestrus (Table 1D), these alterations were not statistically significant. Thus we conclude that the intra-uterine device did not grossly affect this cytosol receptor-associated activity.

(c) *Intra-uterine-device-induced alterations in nuclear oestrogen-receptor content.* At each phase of the cycle, the content of nuclear receptor in the intra-uterine-device-containing horn was approximately equivalent to that in the contralateral control (Table 2A). As also shown in Fig. 1, there was a 5-fold increase in the control, contrasted with a 3-fold increase in the intra-uterine-device-containing horn, when the values for metoestrus were compared with those for pro-oestrus. This observation suggests a lowering of the translocational response to increases in circulating oestrogens in intra-uterine-device-containing horns.

Indeed, when we related the content of nuclear oestrogen receptor to cell number (i.e. per mg of DNA; Table 2B) or to wet weight, the intra-uterine-device-containing horn had a lowered concentration of receptor. This was particularly evident, the differences being statistically significant, at the late dioestrus and pro-oestrus phases. At these two phases, the concentration in intra-uterine-device-containing horns was 65 and 53% respectively of that in control horns. By using a value of 6.5 pg of DNA/cell (Williams & Gorski, 1972) the quantity of receptors per cell was estimated to be (nucleus/cytoplasm) 3150:16300 for treated horns against 4830:17100 for control horns at late dioestrus and 3200:11700 for treated horns against 5980:12600 for control horns at pro-oestrus.

Discussion

The insertion of an intra-uterine device into the uterine horns was followed by an increase in wet

Table 1. *Changes in wet weight, content and oligo(dT)-cellulose binding capacity of the cytosolic oestrogen receptor during the oestrous cycle for intra-uterine-device-containing and control uterine horns*

Four separate groups, each of 30 rats, 88 days of age, fitted with an intra-uterine device in one uterine horn 18 days previously were segregated according to phase of the oestrous cycle. Uteri were removed, weighed and prepared as described in the Experimental section. A saturating concentration (5 nM) of [³H]oestradiol was used to measure the content of cytosol receptor and its ability to bind to oligo(dT)-cellulose as described in the Experimental section. For the cytosol receptor, results are expressed as content per uterine horn or per mg wet wt. The ability of the cytosol receptor to bind to oligo(dT)-cellulose was expressed as a percentage of total cytosolic receptor measured. In each phase the mean $\pm$ S.E.M. is given for four separate determinations each involving four or five animals. Significance of the differences shown was assessed by Student's *t* test: *intra-uterine device significantly different from control ($P < 0.01$); **intra-uterine device significantly different from control ($P < 0.05$); †(d) significantly different from (a) and (b) ($P < 0.01$); ††(d) significantly different from (a), (b) and (c) ($P < 0.001$).

		(a) Pro- oestrus	(b) Oestrus	(c) Metoestrus/ early dioestrus	(d) Late dioestrus
(A) Wet wt. per uterine horn (mg)	Intra-uterine device	268 $\pm$ 12	243 $\pm$ 13	197 $\pm$ 19	217 $\pm$ 27
	Control	176 $\pm$ 11*	181 $\pm$ 19**	123 $\pm$ 6*	137 $\pm$ 9**
(B) Cytosolic oestrogen receptor (fmol/horn)	Intra-uterine device	1368 $\pm$ 236	1105 $\pm$ 101	1361 $\pm$ 118	2013 $\pm$ 65
	Control	939 $\pm$ 92	953 $\pm$ 55	788 $\pm$ 28*	1270 $\pm$ 112*
(C) Cytosolic oestrogen receptor (fmol/mg) wet wt.	Intra-uterine device	5.1 $\pm$ 0.8	4.7 $\pm$ 0.7	6.6 $\pm$ 0.3	9.6 $\pm$ 1.2†
	Control	5.3 $\pm$ 0.3	5.4 $\pm$ 0.4	6.4 $\pm$ 0.3	9.2 $\pm$ 0.4††
(D) Oestrogen-receptor binding to oligo(dT)-cellulose (% of cytosolic receptor)	Intra-uterine device	21.3 $\pm$ 4.8	24.9 $\pm$ 5.2	28.1 $\pm$ 2.5	25.9 $\pm$ 2.4
	Control	27.4 $\pm$ 5.4	25.6 $\pm$ 4.6	31.0 $\pm$ 4.8	29.8 $\pm$ 3.5

Table 2. *Changes in the content of the nuclear oestrogen receptor during the oestrous cycle in intra-uterine-device-containing and control uterine horns*

Measurements of the uterine nuclear content of oestrogen receptor were made, as described in the Experimental section, of the same groups of rats from which were derived the data shown in Table 1. The content of the nuclear receptor is expressed per uterine horn or per mg of DNA. In each phase the mean $\pm$ S.E.M. is given, for four separate determinations, each involving four or five animals. * Intra-uterine device significantly different from control ($P < 0.001$); ** intra-uterine device significantly different from control ($P < 0.01$).

		(a) Pro- oestrus	(b) Oestrus	(c) Metoestrus/ early dioestrus	(d) Late dioestrus
(A) Nuclear oestrogen receptor (fmol/horn)	Intra-uterine device	432 $\pm$ 98	184 $\pm$ 62	137 $\pm$ 44	358 $\pm$ 90
	Control	492 $\pm$ 98	175 $\pm$ 40	104 $\pm$ 30	336 $\pm$ 63
(B) Nuclear oestrogen receptor (fmol/mg of DNA)	Intra-uterine device	821 $\pm$ 85	364 $\pm$ 78	295 $\pm$ 78	805 $\pm$ 119
	Control	1528 $\pm$ 61**	465 $\pm$ 87	414 $\pm$ 110	1234 $\pm$ 133*

weight at all phases of the oestrous cycle. This increase was accompanied by parallel increases in the amount of cytosolic oestrogen receptor, resulting in a constancy of cytosolic receptor concentration in the treated and normal horns. Since receptor concentration is maintained and because one of the distinguishing characteristics of target cells for oestrogen is the presence of specific receptors, the intra-uterine-device-induced increase in uterine weight is probably due to increases in the number of oestrogen-sensitive cells.

The maintenance of the oestrous cycle, despite the presence of the intra-uterine device (in terms of phasic changes in wet weight and receptor content), is consistent with reports that the intra-uterine device does not alter the normal cycle of ovulation, release

of gonadotrophins or corpus-luteum function (Corfman & Segal, 1968). There is some disagreement about the behaviour of the cytosolic oestrogen receptor during the oestrous cycle in normal animals: both increases (Feherty *et al.*, 1970) and decreases (Lee & Jacobson, 1971) at pro-oestrus have been reported. Our results are more consistent with those of White *et al.* (1978), in that the concentration of cytosol receptor was maintained throughout the cycle except for higher concentrations at the late dioestrus phase. This may be explained in part by differences in the concentration of circulating oestrogens in cycles lasting for 4 or for 5 days (Naftolin *et al.*, 1972). White *et al.* (1978) used rats on a 4-day cycle. In our experiments, with rats with a longer cycle, the late dioestrus phase was taken as

day 4 after ovulation when oestrogen concentrations were considerably higher than at the equivalent phase of a 4-day cycle. The uterus is therefore exposed for an increased length of time to oestrogens, which could lead to a greater synthesis of cytoplasmic receptors (and thus increased concentration).

Oestrogens play a decisive part in the normal regulated growth of the uterus. The uterotrophic effect of oestrogens appears to be directly related to an increased nuclear content of oestrogen receptor (Clark *et al.*, 1973; White *et al.*, 1978) leading to increased genetic activity involving DNA, RNA and protein synthesis. However, the increase in uterine wet weight resulting from the insertion of an intra-uterine device was not associated with an increase in the cellular nuclear receptor content. Our observations of a decreased nuclear receptor concentration (Table 2B) in intra-uterine-device-containing horns are therefore inconsistent with the view that the intra-uterine-device-induced tissue growth is the result of a 'hyperoestrogenic' effect (Joshi, 1970) if we accept that the growth-promoting action of oestrogens is mediated by nuclear receptors. Furthermore, the findings of Stock *et al.* (1968), showing that intra-uterine-device-induced growth also occurred in ovariectomized rats, suggest that oestrogens do not play a major role in this type of growth. The unregulated growth may be a non-specific response to tissue distension (Cullen & Harkness, 1968).

It is unlikely that changes in the characteristics of the oestrogen receptor are responsible for the decreased nuclear binding by intra-uterine-device-containing horns, since cytosol and nuclear oestrogen receptors from both control and treated horns had similar dissociation constants. Further, nuclear receptors isolated from both horns had similar stabilities in the exchange assay.

We have considered the possibility that unregulated and excessive tissue growth could lead to a limitation of the vascular supply to the intra-uterine-device-containing horn; this would result in decreased intra-cellular oestrogen concentrations and an apparent decrease in translocation of receptor. It has been shown that the concentration of peroxidase (an oestrogen-induced enzyme) in oestrogen-responsive cells is a direct function of the intracellular oestrogen concentration (Lyttle & DeSombre, 1977). Our studies show that the cellular concentration of peroxidase in intra-uterine-device-containing horns is not lower than in control horns (L. Myatt, M. G. Elder & L. Lim, unpublished work), thus strongly excluding the possibility of limited availability of oestrogen in the treated horn being responsible for decreased translocation.

It is still unclear why the presence of an intra-uterine device should lead to an altered translocation and/or different retention characteristics of the specific receptor for oestrogens. It is pertinent to

note that in the normal uterus the participation of oestrogen is essential to the implantation of the blastocyst (Heald, 1976). The relationship of nuclear content of oestrogen receptors and the receptivity of the rat endometrium for ovo-implantation has also been studied (Martel & Psychoyos, 1976) and it appears that a critical concentration of nuclear receptor is necessary to give the oestrogen-mediated endometrial response necessary for implantation. Clearly, a decreased translocation of oestrogen receptor into the nucleus could interfere with the implantation process. Because of these considerations receptor interactions in intra-uterine-device-containing, infertile and control fertile horns in the first 4 days of pregnancy should be investigated. Such studies should lead to a better understanding of the physiological role of the oestrogen receptor.

We thank the International Planned Parenthood Federation for financial support.

References

- Anderson, J., Clark, J. H. & Peck, E. J., Jr. (1972) *Biochem. J.* **126**, 561–567
- Buller, R. E. & O'Malley, B. W. (1976) *Biochem. Pharmacol.* **25**, 1–12
- Burton, K. (1956) *Biochem. J.* **62**, 315–323
- Clark, J. H., Anderson, J. & Peck, E. J., Jr. (1972) *Science* **176**, 528–530
- Clark, J. H., Anderson, J. & Peck, E. J., Jr. (1973) *Adv. Exp. Med. Biol.* **36**, 15–59
- Corfman, P. A. & Segal, S. J. (1968) *Am. J. Obstet. Gynecol.* **100**, 448–459
- Cullen, B. M. & Harkness, R. D. (1968) *Q. J. Exp. Physiol.* **53**, 33–42
- Doyle, L. L. & Margolis, A. J. (1964) *Fertil. Steril.* **15**, 597–606
- Feherty, P., Robertson, D. M., Waynfirth, H. B. & Kellie, A. E. (1970) *Biochem. J.* **120**, 837–844
- Ginsburg, M., Greenstein, B. D., MacLusky, N. J., Morris, I. D. & Thomas, P. J. (1974) *Steroids* **23**, 773–792
- Heald, P. J. (1976) *J. Reprod. Fertil. Suppl.* **25**, 29–52
- Joshi, S. G. (1970) *Contraception* **2**, 137–148
- King, R. J. B. & Mainwaring, W. I. P. (1974) *Steroid-Cell Interactions*, Butterworth and Co., London
- Labhsetwar, A. P. & Perser, N. (1972) *Acta Endocrinol.* **69**, 583–588
- Laumas, K. R. & Yadava, H. S. (1969) *J. Reprod. Fertil.* **20**, 389–400
- Lee, C. & Jacobson, H. J. (1971) *Endocrinology* **88**, 596–601
- Lyttle, C. R. & DeSombre, E. R. (1977) *Nature (London)* **268**, 337–339
- Martel, D. & Psychoyos, A. (1976) *Endocrinology* **99**, 470–475
- Naftolin, F., Brown-Grant, K. & Corker, C. S. (1972) *J. Endocrinol.* **53**, 17–30
- O'Malley, B. W. & Means, A. R. (1974) *Science* **183**, 610–620

- Parr, E. L. & Segal, S. J. (1966) *Fertil. Steril.* **17**, 648-653
- Scatchard, G. (1949) *Ann. N.Y. Acad. Sci.* **51**, 660-672
- Stock, L., Ledger, W. J. & Campbell, C. (1968) *Fertil. Steril.* **19**, 644-648
- Thrower, S., Hall, C., Lim, L. & Davison, A. N. (1976) *Biochem. J.* **160**, 271-280
- White, J. O., Thrower, S. & Lim, L. (1978) *Biochem. J.* **172**, 37-47
- Williams, D. L. & Gorski, J. (1972) *Acta Endocrinol. (Copenhagen) Suppl.* **168**, 420-452