The effects of oestrogens and progestogens on oestrogen receptors in female rat liver

Winifred MARR*, Murdoch G. ELDER* and Louis LIM†

**Institute of Obstetrics and Gynaecology, Hammersmith Hospital, Du Cane Road, London W12, and*

†*Miriam Marks Department of Neurochemistry, Institute of Neurology, Queen Square, London WC1N 3BG, U.K.*

(Received 29 February 1980)

The administration of oestradiol-17 β or ethynyloestradiol as well as the synthetic progestogen norethisterone acetate resulted in translocation of the oestrogen receptor. Progesterone and the synthetic progestogen (+)-norgestrel were ineffective. The increases in nuclear oestrogen receptor content 1 h after injection of each steroid were similar but differed subsequently. The increase with oestradiol-17 β extended for 3–6 h and for at least 9 h with ethynyloestradiol. With norethisterone acetate, nuclear content was still increased after 24 h. Oestrogen injection resulted in cytosol receptor depletion and a 'deficit' in receptor content extending for 6 h, whereas norethisterone acetate-induced translocation was quantitative. With injections of norethisterone acetate + ethynyloestradiol the increase at 1 h and retention of the nuclear receptors were similar to that with norethisterone acetate alone. In contrast, the depletion of cytosol receptor and its restoration were similar to that seen with ethynyloestradiol alone, suggesting that norethisterone acetate did not interfere with the oestrogen receptor replenishment. Specific binding *in vitro* of [³H]oestradiol-17 β in liver cytosols was inhibited by (+)-norgestrel and norethisterone acetate, but not progesterone, at concentrations of 10–100 μ M. Nuclear receptors present after norethisterone acetate injection bound oestrogen with high affinity ($K_d = 1.52$ nM), similar to receptors of oestrogen-injected animals. In the uterus, differential retention of nuclear receptors in response to oestrogens is associated with different cellular responses. The differences in the response of the receptor system in liver to the various steroids suggests that the corresponding tissue responses may also be dissimilar. These results are discussed in relation to the problems of liver dysfunction in oral-contraceptive users.

Mammalian liver is known to be responsive to the actions of oestrogens (Song & Kappas, 1968) and the direct effects of the hormone have been demonstrated by using perfused liver preparations (Nasjlette & Masson, 1972). The direct effects of oestrogens on the liver may be mediated by oestrogen receptors (Eisenfeld *et al.*, 1976; Aten *et al.*, 1978) as with other target tissues such as uterus (King & Mainwaring, 1974).

We have reported the presence of high-affinity specific receptors for oestrogens in both the cytosol and nuclear fractions of mature female rat liver and demonstrated receptor translocation to the nucleus by administration of high doses of oestradiol-17 β and ethynyloestradiol (Marr *et al.*, 1980).

In the uterus the extent of the cellular response to oestrogens appears to be governed by the concentration of nuclear receptors. Retention of these

receptors for at least 4–6 h after oestradiol-induced translocation is requisite for true uterine growth; nuclear receptors retained for shorter terms appear to be associated with limited cellular responses (Clark *et al.*, 1972, 1973). The retention in liver of nuclear receptors for periods longer than 1 h in response to injected oestrogens has not been investigated and the role of nuclear oestrogen receptors in relation to the control of liver function remains unknown.

Progesterone has long been considered an antagonist of oestrogen action in uterus and other target tissues (Lerner, 1964; Hsueh *et al.*, 1976). However, the administration of certain synthetic progestogens (including norethisterone) with oestrogen to women does not inhibit oestrogen-induced alterations in liver protein synthesis (Musa *et al.*, 1967). Furthermore, these synthetic progestogens,

which are commonly used in oral-contraceptive preparations, can themselves induce changes in the liver synthesis of certain proteins such as plasminogen and fibrinogen. These effects can be either similar or dissimilar to those resulting from administration of oestrogens (Barbarosa *et al.*, 1971).

In the present paper we have examined the response of the oestrogen receptor system in the rat liver to injections of oestradiol-17 β or ethynyl-oestradiol, which induce receptor translocation, to determine whether there were similarities in the receptor systems in liver and uterus, particularly with regard to the presence of long-term nuclear receptors. As we are particularly interested in the problems of liver dysfunction caused by oral contraceptives, we have also included the synthetic progesterone (+)-norgestrel and norethisterone acetate, as well as progesterone, to determine their effect on the liver oestrogen receptor system.

Experimental

Materials

[2,4,6,7(n)-³H]Oestradiol-17 β (sp. radioactivity 100 Ci/mmol) was supplied by The Radiochemical Centre, Amersham, Bucks., U.K. Sephadex LH-20 was from Pharmacia (G.B.) Ltd., London W.5, U.K. Butyl-PBD [5-(biphenyl-4-yl)-2-(4-t-butylphenyl)-1-oxa-3,4-diazole] was from Ciba, Horsham, Sussex, U.K. Sigma (London) Chemical Co., Poole, Dorset, U.K., supplied 17 α -ethynyl-oestradiol (17 α -ethynyl-oestra-1,3,5-triene-3,17 β -diol) (>99% pure), oestradiol-17 β [oestra-1,3,5(10)-triene-3,17 β -diol] (>99% pure), progesterone (pregn-4-ene-3,20-dione) and norethisterone acetate (17 α -ethynyl-17 β -hydroxy-oestr-4-en-3-one 17 β -acetate) (>99.5% pure). (+)-Norgestrel M20 [(+)-13 β -ethyl-17-hydroxy-18,19-dinor-17 α -pregn-4-en-20-yn-3-one] was supplied by Schering A.G., Berlin/Bergkamen, Germany. Purity of the relevant steroids was determined by t.l.c. with several mixed-solvent systems.

Animals

Female Wistar rats (80–85 days old) were bred in our laboratories at the Institute of Neurology. Four to six rats comprised each experimental group and animals were decapitated.

Injections

All steroids were dissolved in propylene glycol except (+)-norgestrel, which was administered as a suspension. Subcutaneous injections of 0.1 ml constant volume were given into the nape of the neck, using a separate site for each injection when combined steroids were administered.

Methods

The preparation of the liver fractions and measurement of the cytosol and nuclear oestrogen receptors was carried out as previously described (Marr *et al.*, 1980). Briefly, the specific receptor content of cytosols was determined by incubating 0.2 ml portions in duplicate with 10 nM-[³H]oestradiol with and without 0.5 μ M-diethylstilboestrol for 18 h at 0–4°C. The samples were then chromatographed on columns of Sephadex LH-20 before measurement of bound radioactivity. For nuclear receptor measurements 0.2 ml portions of nuclear suspension were incubated in triplicate with 10 nM-[³H]oestradiol in the absence and presence of 0.5 μ M-diethylstilboestrol at 37°C for 30 min. The specifically bound radioactivity was measured by liquid-scintillation counting.

Tissue wet weight

The wet weight of liver was measured in each experimental animal and found not to vary under any of the steroid regimens used in the present paper.

Protein and DNA determinations

The protein content of the cytosol fractions was measured by the method of Lowry *et al.* (1951). The DNA content of the nuclear fractions was determined by the Schmidt–Thannhauser procedure with the modifications recommended by Munro & Fleck (1966).

Results

Liver cytosol and nuclear receptor measurements after injection of oestrogens

Nuclear receptor retention and cytosol receptor replenishment. Rats were injected with either 10 μ g of ethynyl-oestradiol or 50 μ g of oestradiol-17 β to effect maximal receptor translocation (Marr *et al.*, 1980) and the receptor content and distribution was measured at various times after injection. The patterns of nuclear receptor retention and cytosolic receptor replenishment were different for the two oestrogens (Fig. 1). With oestradiol-17 β the retention of nuclear receptors was transient and the cytosol receptor content was restored to control values within 6 h. Injection of ethynyl-oestradiol, however, resulted in a more prolonged retention of nuclear receptors with 60% of the receptor content at 1 h still present at 6 h. The corresponding cytosol receptor replenishment was slower than that induced by oestradiol-17 β . In both cases at 1 h there was a net deficit in the total (cytosol + nuclear) receptor content.

In every experiment in the present paper the cytosol protein content of the steroid-injected groups

Fig. 1. The effects of administered oestrogens on oestrogen receptor content and distribution

Groups of four to six rats were each injected with $10\mu\text{g}$ of ethynyloestradiol or $50\mu\text{g}$ of oestradiol- 17β and killed at different times after injection. Controls received vehicle alone. The cytosol and nuclear oestrogen receptor contents were determined as described in the Experimental section. Results throughout are expressed as means $\pm$ S.E.M. for data from separate experiments on three individual groups, unless stated otherwise. The absence of error bars indicates a mean value for data from two experiments. Symbols: $\circ$, controls; $\bullet$, oestrogen-injected. Nuclear (a and c) and cytosol (b and d) receptor contents are shown for oestradiol- 17β -injected groups (a and b) and ethynyloestradiol-injected groups (c and d). The 6 h values involve results from six separate determinations.

was found to be similar to that of control groups of 65–78 mg/g wet weight. Similarly the DNA content of the nuclear fractions of the steroid-injected animals did not differ from the controls (1.92–2.2 mg/g wet weight).

Fig. 2. Displacement of $[^3\text{H}]$ oestradiol binding to liver cytosol by oestrogens and progestogens

Portions of cytosol (0.2 ml) were incubated with 10 nM $[^3\text{H}]$ oestradiol and various concentrations of non-radioactive oestrogens and progestogens for 18 h at $0-4^\circ\text{C}$. Total bound radioactivity was determined and the results are expressed as the mean value of triplicate measurements, which agreed to within 10% [control values obtained by incubating portions with 10 nM $[^3\text{H}]$ oestradiol alone were similar to values obtained with 0.1 nM non-radioactive steroids present].

The effects of progestogens on oestrogen receptors

Because of reports that synthetic progestogens also affect liver function, the effects on oestrogen receptors of administering two progestogens commonly employed in oral contraceptives, (+)-norgestrel, a gonane, and norethisterone acetate, an oestrane, were investigated.

(a) *Specificity of cytosol oestrogen receptors.* To determine the interaction of these progestogens with the oestrogen receptor, we first tested the ability of several oestrogens and progestogens to inhibit binding *in vitro* of $[^3\text{H}]$ oestradiol to liver cytosol. Low concentrations of the oestrogens inhibited $[^3\text{H}]$ oestradiol binding, whereas higher concentrations of (+)-norgestrel and norethisterone acetate were required to produce a similar effect (Fig. 2). Progesterone did not inhibit $[^3\text{H}]$ oestradiol binding to liver cytosol. At higher concentrations ($0.01-10\mu\text{M}$) oestradiol- 17β was slightly less potent an inhibitor of $[^3\text{H}]$ oestradiol binding than the synthetic oestrogens.

(b) *The effects in vivo of progestogens on oestrogen receptors.* To test for possible effects *in vivo* of these progestogens on oestrogen receptors, animals were injected with various doses of (+)-norgestrel (0.25–10 mg/rat), norethisterone acetate (0.01–10 mg/rat) and progesterone (1–10 mg/rat) and the nuclear and cytosol oestrogen receptor content determined 1 h after injection. The receptor content and distribution in (+)-norgestrel- and progesterone-injected groups was found to be similar to controls (result not shown). Hence, administra-

Fig. 3. Changes in oestrogen receptor content in response to administration of norethisterone acetate. Different doses of norethisterone acetate (10 µg–10 mg/rat) were injected into groups of animals that were killed 1 h later and the nuclear and cytosol oestrogen receptor contents determined. In this and subsequent Figures, hatched bars represent nuclear receptor, open bars cytosol receptor.

tion of these two progestogens did not result in translocation.

With norethisterone acetate (Fig. 3) there was quantitative translocation of receptor. Doses of 250 µg–10 mg elicited increases in nuclear oestrogen receptor content of approx. 0.7 pmol/g of tissue and a decrease in cytosol receptor content of 0.5–0.8 pmol/g of tissue. In contrast, injections of high doses of oestrogens caused a similar increase in nuclear receptor content, which was accompanied by a marked depletion of cytosol receptor content of about 2.5 pmol/g of tissue 1 h after injection.

(c) *The effects of (+)-norgestrel and norethisterone acetate on oestrogen receptors 6 h after injection.* It is possible that the lack of interaction *in vivo* of (+)-norgestrel (or its metabolites) with oestrogen receptors is due not only to differences in the liver metabolism of the two synthetic progestogens, but to differences in absorption/clearance, particularly as (+)-norgestrel is administered as a suspension. To determine whether (+)-norgestrel would induce receptor translocation with an increased time of exposure to the steroid, the receptor content and distribution were measured 6 h after injection. It was found that only the injection of norethisterone acetate but not (+)-norgestrel resulted in increased nuclear oestrogen receptor content. Despite binding *in vitro* of (+)-norgestrel to oestrogen receptors in cytosol, this progestogen and/or its metabolites did not interact with oestrogen receptors *in vivo*.

Fig. 4. The effects of combined oestrogen/progestogen administration on oestrogen receptor content 6 h after injection.

Combined injections of different doses of norethisterone acetate (NorAc) (1 mg or 5 mg/rat) of (+)-norgestrel (Norg) (0.25 or 1 µg/rat) + 10 µg of ethinyloestradiol (EE₂) or injections of 10 µg of ethinyloestradiol alone were given and the animals were killed 6 h later for determination of nuclear (hatched bars) and cytosol (open bars) oestrogen receptor content. The protocol is described in the Experimental section.

(d) *The effects of combined oestrogen/progestogen injections on oestrogen receptors 6 h after injection.* To investigate the effects of combined administration of oestrogens and progestogens on the oestrogen receptors, measurements of the receptors were made 6 h after injection of different doses of (+)-norgestrel or norethisterone acetate together with 10 µg of ethinyloestradiol. From Fig. 4 it is seen that the cytosol receptor contents were similar in all the steroid-injected groups. Furthermore, the nuclear receptor contents of the norgestrel-plus-ethinyloestradiol-injected animals were similar to those injected with ethinyloestradiol alone. In the norethisterone acetate-plus-ethinyloestradiol-injected groups, the increase in nuclear receptor content was greater than that resulting from ethinyloestradiol injection alone.

These results show that (+)-norgestrel did not interfere with the interaction of oestrogen with its receptors, nor with the translocation process nor the increase in nuclear receptor content at 6 h. With norethisterone acetate, however, the higher nuclear receptor contents confirmed the oestrogenic effect of this progestogen, whereas the depleted cytosol receptor content possibly resulted from the oestrogen component of the injected combination.

Operations of the oestrogen receptor system after injection of norethisterone acetate

(a) *The binding affinity of nuclear oestrogen receptors in norethisterone acetate-injected animals.* The dissociation constant (K_d) of the nuclear oestrogen receptors present after injection of 1 mg of norethisterone acetate was determined as shown in Fig. 5. The Scatchard (1949) analysis of oestrogen binding to these nuclear fractions showed that a single class of binding sites was present with high affinity for oestrogen binding [$K_d = 1.52 \pm 0.19$ nM ($n = 3$)], similar to oestrogen receptors in livers of normal and oestrogen-injected rats. This result shows that the nuclear receptor population measured after injection of norethisterone acetate was derived most probably from oestrogen receptor translocated from the cytoplasm.

(b) *Nuclear receptor retention and cytosol receptor replenishment on injection of norethisterone acetate.* To compare the effects of norethisterone acetate injection on the operations of the oestrogen receptor system with those of oestrogens, animals were each injected with 5 mg of norethisterone acetate and the retention of nuclear oestrogen receptors and the replenishment of cytosol receptors was investigated.

The nuclear receptor content was elevated above control values at 1 h and 6 h (Fig. 6), with 50%

of the receptor content at 1 h still present after 24 h. The corresponding cytosol receptor contents indicated a quantitative depletion of receptor, which was restored to control values within 24 h.

These patterns differ from those resulting from oestradiol-17 β and ethnyloestradiol injections; in particular the elevated nuclear receptor content at 24 h after injection of 5 mg of norethisterone acetate appeared to be similar to sustained increases in uterine nuclear receptor content in response to antioestrogens such as nafoxidine, compound CI-628 or clomiphene (Clark *et al.*, 1974, 1977). These compounds cause uterine growth and nuclear receptor retention that exceeds that induced by oestradiol. However, there was no increase in liver wet weight at 24 h in response to injection of norethisterone acetate.

(c) *The effects of combined norethisterone acetate plus ethnyloestradiol injections on oestrogen receptors.* The effects of combined injections of 5 mg of norethisterone acetate and 10 μ g of ethnyloestradiol on the patterns of nuclear and cytosol receptor content are shown in Fig. 7.

The increase in nuclear receptor content at 1 and 24 h was similar to that seen on injection of norethisterone acetate alone. However, at 6 h the elevated nuclear content was in excess of that seen on injection of norethisterone acetate alone and of ethnyloestradiol alone, suggesting a possible additive effect of the combined injections at this particular time. The cytosol receptor contents,

Fig. 5. Scatchard analysis of oestrogen binding to nuclear fractions 6 h after administration of norethisterone acetate

Groups of rats were each injected with 1 mg of norethisterone acetate and killed 6 h later. Samples of nuclear suspension (0.2 ml) were incubated in triplicate at 37°C for 30 min with various concentrations of [3 H]oestradiol (0.1–20 nM) with or without a 50-fold excess of diethylstilboestrol at each concentration and the specific binding was determined. The dissociation constant (K_d) was obtained from the plot of steroid bound against the ratio of bound versus free steroid in three separate experiments.

Fig. 6. Oestrogen-receptor content and distribution after administration of norethisterone acetate

Norethisterone acetate (5 mg/rat) was administered and the groups were killed 1, 6 or 24 h after injection. Oestrogen-receptor measurements in cytosol (open bars) and nuclear (hatched bars) fractions were made at each time point. Abbreviations used: NorAc, norethisterone acetate.

Fig. 7. The effect of combined injections of norethisterone acetate and ethynyloestradiol on oestrogen receptor content and distribution

Each rat was injected with 5 mg of norethisterone acetate + 10 µg of ethynyloestradiol, as described in the Experimental section. The animals were killed 1, 6 and 24 h after injection and the cytosol (open bars) and nuclear (hatched bars) oestrogen receptor contents were determined. Abbreviations used: NorAc, norethisterone acetate; EE₂, ethynyloestradiol.

however, were similar to those measured after administration of ethynyloestradiol alone, showing a rapid depletion of cytosol receptor at 1 h and a restoration to control values at 24 h.

Discussion

There were marked differences in the pattern of nuclear retention of the oestrogen receptors on injection of the two oestrogens and norethisterone acetate despite a uniform increase in nuclear content 1 h after injection of each steroid. This uniform increase suggests a saturation of nuclear acceptor sites or a rapid 'processing' involving deactivation and/or removal of nuclear receptors in excess of the concentration present within 1 h of the injection (0.8–1.0 pmol/g of tissue). However, the long-term receptor content, as defined by those still present 6 h after steroid administration (Clark *et al.*, 1974), was substantially increased only by ethynyloestradiol ($P < 0.01$; Fig. 1c) and norethisterone acetate ($P < 0.005$; Fig. 6). A major proportion of these long-term receptors (60%) was still present 24 h after norethisterone acetate administration (Fig. 6).

The administration of norethisterone acetate resulted in an increase in nuclear content of the

oestrogen receptor, which quantitatively reflected cytosol receptor depletion. In contrast the administration of the oestrogens resulted in cytosol receptor depletion far in excess of the nuclear receptor increase. This 'deficit' in receptor content, although less marked, is also observed on administration of lower doses of oestrogen that effect a limited translocation (Marr *et al.*, 1980). The loss of receptor may be due in part to rapid processing of the nuclear receptors (Aten *et al.*, 1978). It is unlikely that the 'loss' results from underestimation of the oestrogen-translocated nuclear receptor, since in all cases our nuclear exchange procedure (Marr *et al.*, 1980) involved incubation at 37°C for 30 min with 10 nM-[³H]-oestradiol-17β; this concentration is in excess of the K_d values of the nuclear oestrogen receptors (1–3 nM; Fig. 5 and Marr *et al.*, 1980) present after administration of all three steroids and thus sufficient to ensure quantitative exchange. However, it is also possible that the receptors are shunted to other cellular compartments, inactivated or destroyed by proteinases in the cytoplasm. Alternatively, receptors in the cytosol might be occupied with endogenous steroid and hence are undetectable in our cytosol receptor assay. This is unlikely, since Aten *et al.* (1978) demonstrated that the majority of cytosol receptors present after injection of ethynyloestradiol (100 µg) were unoccupied on the basis of exchange assays on (NH₄)₂SO₄ precipitates of liver cytosols.

With combined injections of norethisterone acetate and ethynyloestradiol the increase at 1 h in the nuclear receptor content was similar to that obtained with the single steroid injections, although the receptors might be translocated with different ligands arising from injection of the oestrogen and progestogen. This finding supports the proposal of saturable nuclear 'acceptor' sites.

The effects of the administration of the various steroids on the total (i.e. nuclear + cytosol) receptor content are summarized and are compared in Fig. 8. The administration of oestrogen, whether singly or in combination with norethisterone acetate, was always accompanied by a resultant net deficit of receptor 1 h later. This deficit was not observed when norethisterone acetate alone was administered. There are differences in the replenishment mechanism after administration of the different oestrogens since a net deficit of receptor is still observed 6 h after administration of ethynyloestradiol, but not of oestradiol-17β.

Norethisterone acetate-induced receptor translocation *in vivo* could be due to a metabolite of the progestogen, which interacted with the oestrogen receptor. Ethynyloestradiol has been identified as a metabolite of norethisterone in the rat and human (Braserton *et al.*, 1977) although this conversion may be limited in humans (Breuer, 1970; Fotherby,

Fig. 8. The total (cytosol + nuclear) oestrogen receptor content after administration of the oestrogens, of norethisterone acetate and of norethisterone acetate + ethynyl-oestradiol

The total (cytosol + nuclear) oestrogen receptor content was determined for the groups of rats injected with (a) oestradiol-17 β (50 μ g/rat), (b) ethynyl-oestradiol (10 μ g/rat), (c) norethisterone acetate (5 mg/rat) or (d) combined injections of norethisterone acetate (5 mg/rat) + ethynyl-oestradiol (10 μ g/rat). The receptor measurements at 1, 6 and 24 h after injection are shown. Values for controls injected with vehicle alone are shown in (e). These values are derived from examination of data given in previous Figures.

1974). However, the marked differences in the responses of the oestrogen receptors to ethynyl-oestradiol and norethisterone acetate injections suggest that the ligand involved in this progestogen-induced translocation is not ethynyl-oestradiol itself but possibly another metabolite.

In the rat uterus, progesterone exerts its oestrogen antagonistic effect by interfering with cytosol

oestrogen receptor replenishment, possibly via progesterone receptor mediation (Hsueh *et al.*, 1976). Liver progesterone receptors have not as yet been reported. Nevertheless, it appears that norethisterone acetate does not exhibit the characteristics of an oestrogen antagonist, whether or not such progestogen receptors occur, since replenishment of the cytosol oestrogen receptors in the rat liver was unaffected by norethisterone acetate (Fig. 7). Changes in plasma proteins that result from oestrogen administration in women are also not prevented by the inclusion of synthetic progestogens (including norethisterone) in doses used in oral-contraceptive preparations (Musa *et al.*, 1967; Laurell *et al.*, 1968). Furthermore, the reported oestrogenic effects of norethisterone (Edgren, 1978; Edgren *et al.*, 1968), including observations that high doses of the progestogen may increase plasma concentrations of plasminogen and corticosteroid-binding globulins (Kalkhoff, 1976), are in keeping with our observations of an induced translocation of the oestrogen receptor.

There was little or no effect of (+)-norgestrel on the distribution of oestrogen receptors *in vivo*, although (+)-norgestrel was bound *in vitro* to oestrogen receptors in liver cytosol with higher affinity than norethisterone acetate. (+)-Norgestrel undergoes some metabolism to a weak oestrogen, ethynyl-18-methyloestradiol, in humans (Fotherby, 1974). It is possible that metabolism of (+)-norgestrel *in vivo* prevented its interaction with liver oestrogen receptors in the rat. Norgestrel is reported to attenuate many oestrogen-induced effects on liver protein synthesis in women taking combined-type oral contraceptives (Briggs & Briggs, 1973). This oestrogen antagonistic effect of norgestrel suggests that the compound has progestogenic effects on liver. However, we have not studied the effects of natural progesterone or (+)-norgestrel on the replenishment of oestrogen receptors in liver.

The characteristics of the oestrogen receptor of liver are similar to those of uterus in that both the cytosol and nuclear receptors are of high specificity and affinity and limited capacity (Marr *et al.*, 1980). Nevertheless, there are differences in the operation of the receptor system, since large doses of the oestrogens are required to promote translocation in the liver [results reported in the present paper and by Marr *et al.* (1980)]. This requirement may explain the absence in liver of cyclic changes in nuclear receptor content (Marr *et al.*, 1980) that occur in other target tissues, including the uterus (White *et al.*, 1978). Nuclear binding of receptor was also found to be restricted in the liver, even with high doses of oestrogens. A major difference between the response of the uterus and the liver is that no increases in liver DNA or protein content ever occurred on administration of any of the steroids.

Some of the differences observed between liver and uterus on steroid administration may be the result of metabolizing enzymes in liver which serve to regulate the interaction of steroids with the oestrogen receptor. Metabolic conversion of norethisterone acetate in the liver may also explain its ability to promote translocation of the oestrogen receptor *in vivo*. The effects *in vivo* of this progestogen on uterine cytosol oestrogen receptors have not been reported, although it has been shown that uterine cytosol oestrogen receptors bind the progestogen *in vitro* with a low K_d of $<0.1\mu\text{M}$ (Terenius, 1974; Kordelaar *et al.*, 1975). The results reported in the present paper on the oestrogenic effect of the progestogen emphasize the need for continued investigations on the effects of this progestogen on liver (and uterus) oestrogen receptors.

It is possible that certain aspects of liver dysfunction reported in oral-contraceptive users involve interaction of synthetic sex steroids with a similar receptor system, although at this stage it is difficult to extrapolate our studies in the rat to the human situation because of species differences. However, in the design of oral contraceptives or in hormonal replacement therapy, care should be taken to use a low dose of oestrogen and a progestogen component that has minimal interaction with oestrogen receptors, in an effort to decrease the undesirable side-effects of sex steroids on liver function.

We thank Mark Habershon for his expert help with the animals. This work has been supported in part by a grant from Wyeth International (U.S.A.), which is gratefully acknowledged. L. L. thanks the Brain Research Trust for the provision of certain laboratory equipment.

References

- Aten, R. F., Weinberger, M. J. & Eisenfeld, A. J. (1978) *Endocrinology* **102**, 433–442
- Barbarosa, J., Seal, U. S. & Doe, P. R. (1971) *J. Clin. Endocrinol.* **32**, 547–554
- Braserton, W. E., Lin, T. G., Mills, M. T., Ellegood, J. O. & Mahesh, V. B. (1977) *J. Steroid Biochem.* **8**, 9–18
- Breuer, H. (1970) *Lancet* **ii**, 615–616
- Briggs, M. & Briggs, M. (1973) *Biochem. Pharmacol.* **22**, 2277–2281
- 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
- Clark, J. H., Anderson, J. & Peck, E. J., Jr. (1974) *Nature (London)* **251**, 446–448
- Clark, J. H., Paszko, Z. & Peck, E. J., Jr. (1977) *Endocrinology* **100**, 91–96
- Edgren, R. A. (1978) *Norethindrone: The First Three Decades*, Syntax Laboratories Inc., pp. 15–36
- Edgren, R. A., Jones, R. C., Clancy, D. P. & Nagra, C. L. (1968) *J. Reprod. Fertil. Suppl.* **5**, 13–45
- Eisenfeld, A. J., Aten, R., Weinberger, M., Haselbacher, G., Halpern, K. & Krakoff, L. (1976) *Science* **191**, 862–866
- Fotherby, K. (1974) *The Second International Nor-gestrel Symposium*, pp. 30–34, The Royal College of Physicians, London
- Hsueh, A. J. W., Peck, E. J., Jr. & Clark, J. H. (1976) *Endocrinology* **98**, 438–444
- Kalkhoff, R. K. (1976) *Excerpta Med. Found. Int. Congr. Ser.* **402**, 360–364
- King, R. J. B. & Mainwaring, W. I. P. (1974) *Steroid Cell Interactions*, pp. 288–295, Butterworths, London
- Kordelaar, J. M. G., Vermorken, A. J. M., De Weerd, C. J. M. & Van Rossum, J. M. (1975) *Acta Endocrinol. (Copenhagen)* **78**, 165–179
- Laurell, C. B., Kullander, S. & Thorell, J. (1968) *J. Clin. Lab. Invest.* **21**, 337–341
- Lerner, L. J. (1964) *Recent Prog. Horm. Res.* **20**, 435–490
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
- Marr, W., White, J. O., Elder, M. & Lim, L. (1980) *Biochem. J.* **190**, 17–25
- Munro, H. N. & Fleck, A. (1966) *Methods Biochem. Anal.* **14**, 113–176
- Musa, B. U., Doe, R. P. & Seal, U. S. (1967) *J. Clin. Endocrinol.* **27**, 1463–1469
- Nasjlette, A. & Masson, G. M. G. (1972) *Circ. Res.* **30–31**, Suppl. 2, 187–198
- Scatchard, G. (1949) *Ann. N.Y. Acad. Sci.* **51**, 660–672
- Song, C. S. & Kappas, A. (1968) *Vitam. Horm. (N.Y.)* **26**, 147–195
- Terenius, L. (1974) *Steroids* **23**, 909–919
- White, J. O., Thrower, S. & Lim, L. (1978) *Biochem. J.* **172**, 37–47