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Title: Mechanism of Action of Exogenous Estrogen in the Ovine Endometrium During the Mid-Luteal Phase of the Estrous Cycle

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The mechanism of action of exogenous estradiol in the ovine endometrium during the mid-luteal phase of the estrous cycle was investigated by (1) determining whether this steroid is capable of provoking increases in the concentrations of cytoplasmic and nuclear progesterone and estrogen receptors, and (2) examining the temporal relationships between changes in RNA polymerase activities and nuclear-bound estrogen.

Mature ewes received a single intramuscular injection of 500 μg estradiol-17β on Day 11 and/or 12 of the estrous cycle and were necropsied at various intervals post-treatment. Total cytoplasmic and nuclear progesterone and estrogen receptors were determined by exchange assay using [^{3}H]17,21-dimethyl-19-nor-4,9-pregnadiene-3,20-dione (R5020) and [^{3}H]estradiol, respectively. The in vitro incorporation of [^{3}H]-thymidine into DNA was used as a corollary measure of uterine response to estradiol. Activities of RNA polymerase I and II were determined by [^{3}H]UTP incorporation into RNA during incubation of endometrial

nuclei in the absence and presence of α-amanitin, respectively. Jugular blood serum was quantified for progesterone by radioimmunoassay.

The equilibrium dissociation constant (K_d) for the progesterone-receptor complex and the maximum concentration of receptors in endometrial cytosol and nuclei were determined to be 1.85 nM and 1130 fmole/mg DNA and .71 nM and 200 fmole/mg DNA, respectively. No change in cytoplasmic progesterone receptors was detected by 24 or 48 h after a single injection of estradiol. Two sequential injections of hormone were necessary to stimulate increases ($P<.01$) in cytoplasmic progesterone receptors and DNA synthesis. Concentrations of nuclear progesterone receptors and serum progesterone did not differ among control and treated ewes. A transient increase in the concentration of nuclear-bound estrogen occurred at 1 h ($P<.01$) after the second of two sequential injections of estradiol which stimulated increases ($P<.01$) in the activity of RNA polymerase I at 6, 12 and 24 h post-treatment. The activity of RNA polymerase II tended to increase with time, but the differences among intervals were not significant statistically.

Results from these studies indicate that exogenous estradiol was able to elicit increases in endometrial ctyoplasmic progesterone receptors and RNA polymerase activities in the presence of relatively high concentrations of endogenous progesterone. These effects of estradiol involved the interaction of estradiol-receptor complexes with the nucleus. The estradiol-induced increase in RNA polymerase I activity may lead to RNA synthesis essential for promoting the observed increases in cytoplasmic progesterone receptors and/or synthesis of the uterine luteolysin.

Mechanism of Action of Exogenous Estrogen in the Ovine Endometrium During the Mid-Luteal Phase of the Estrous Cycle

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MECHANISM OF ACTION OF EXOGENOUS ESTROGEN IN THE OVINE ENDOMETRIUM DURING THE MID-LUTEAL PHASE OF THE ESTROUS CYCLE

REVIEW OF LITERATURE

Introduction

Uterine function during various reproductive states of the animal has been shown to be largely under the control of estrogen and progesterone. Subsequent to the discovery of estrogen from porcine ovarian follicular fluid (Allen and Doisey, 1923) and progesterone from corpora lutea (Corner and Allen, 1929), laboratory synthesis of both hormones in mass quantities enabled numerous studies to be conducted to elucidate the action of these steroids on the uterus.

Estrogen action on the uterus is generally stimulatory. The characteristic uterotropic responses elicited by estrogen have been classified as "early" (within the first 6 h after estrogen administration) and "late" (between 12 and 36 h post-treatment) events (Clark et al., 1973). Some of the more notable early responses observed include the following biochemical and metabolic events: histamine mobilization (Spaziani and Szego, 1958), hyperemia (Szego and Roberts, 1953), water imbibition (Astwood, 1938), increased glucose oxidation (Nicolette and Gorski, 1964), increased lipid synthesis (Aizawa and Mueller, 1961), DNA-dependent RNA synthesis (Hamilton et al., 1968), synthesis of a specific induced protein (Barnea and Gorski, 1970), and increases in RNA polymerase I and II activities (Noteboom and Gorski, 1963; Glasser et al., 1972). Many investigators

have proposed that these early uterotropic events are preparatory and supportive of the late responses. Late responses to estrogen were observed to be continued stimulation of RNA polymerase I and II activities (Glasser et al., 1972; Glasser and Spelsberg, 1973), increased general protein synthesis (Mueller, 1953), increased uterine dry weight (Astwood, 1939a), and stimulation of DNA synthesis (Epifanova, 1966); all of which reflect uterine cellular hypertrophy and hyperplasia.

The dependence of the uterus on progesterone was first noted as "progestational proliferation" of the endometrium; a physiological state considered to be necessary for implantation and development of the embryo (Corner and Allen, 1929). Subsequent studies established that blastocyst implantation and maintenance of pregnancy in the ovariectomized animal could be achieved by sufficient quantities of exogenous progesterone (Allen and Corner, 1930; Chang, 1951; Lyons, 1943; Pincus et al., 1956). Astwood (1939b) discovered another physiological effect of progesterone on the uterus, namely deciduoma formation in pseudopregnant ovariectomized rats. Most of the early results of progestational action in the uterus were obtained utilizing ovariectomized or immature animals primed with estrogen and for the most part were restricted to the study of histological preparations of the uterus. The most prominent uterine responses to progesterone treatment were observed to be stimulated secretion by epithelial cells, increased length of tubular glands, and enhanced uterine growth (McPhail, 1934; McGinty et al., 1939). Hooker and Forbes (1947) also noted progesterone-induced hypertrophy of stromal nuclei in the

endometrium. Progesterone has been found to cause myometrial quiescence during pregnancy (Csapo, 1956; Csapo and Wiest, 1969) by suppressing spontaneous myometrial contractions and decreasing electrical coupling between myometrial cells (Ichikawa and Bortoff, 1970). Progesterone also has been demonstrated to regulate the activities of some endometrial enzymes. More specifically, progesterone has been shown to increase carbonic anhydrase (Lutwak-Mann, 1955), phosphatase (Murdock and White, 1966), glucose-6-phosphate dehydrogenase and isocitric dehydrogenase (Lerner et al., 1966) activities and to inhibit estradiol-induced pyruvate kinase activity (DeAusa et al., 1968). More recently, administration of progesterone has been found to promote collagen synthesis through an increased activity of protocollagen hydroxylase (Kao et al., 1969).

Prior to the late nineteen sixties considerable knowledge had been accumulated concerning the physiological effects of exogenous estrogen and progesterone on their target organs. The subsequent widespread use of the radioimmunoassay to quantify hormones in systemic blood during various reproductive states confirmed the role of these steroids in regulating target organ function as established by early investigators. More importantly it became obvious that these hormones were present in blood in relatively minute quantities. Investigators began to question the mechanism(s) by which circulating steroids were able to "recognize" and act on their appropriate target cells to evoke a characteristic biological response. Only in the past decade has the fusion of the fields of endocrinology and molecular biology led to insight into the mechanism of action of steroid hormones and regulation of target cell function.

Mechanism of Action of Progesterone and Estrogen in the Uterus

Paramount to the elucidation of steroid hormone action was the demonstration of differential accumulation and retention of tritium-labeled estradiol of a high specific activity in the uterus and other target organs (Jensen and Jacobson, 1962). This observation suggested that accumulation occurred without metabolic conversion of estradiol. Specific retention of labeled progesterone by target tissues has been measured in only a few cases because of the extensive conversion of progesterone into polar metabolites (Falk and Bardin, 1970; Leavitt and Blaha, 1972). The first clue to the nature of steroid retention by target cells was provided by Toft and Gorski (1966) who demonstrated the presence of a soluble protein capable of binding labeled estradiol with high affinity in the cytoplasmic fraction of the homogenized rat uterus. This class of proteins is now referred to as "receptors," and research conducted since this initial observation has verified their existence in target tissues of a number of species.

Subsequent experiments led to the elucidation of a "two-step" model of steroid action whereby the steroid is bound rapidly and with great affinity to its cytoplasmic receptor after which the steroid-receptor complex is translocated to the nucleus (Gorski et al., 1968; Jensen et al., 1968). These pioneering studies laid the groundwork for the generally accepted dogma that the characteristic effects elicited by progesterone and estrogen on the uterus are mediated by the genome.

Properties of the Estrogen Receptor

Utilization of sucrose gradient ultracentrifugation with the addition of low salt (less than 0.1 M KCl) has enabled separation of the high affinity estrogen receptor from the low affinity, non-specific binding components (Toft and Gorski, 1966) that comprise approximately 10 to 20 percent of the total available estrogen binding sites (Katzenellenbogen et al., 1973). This method also has been useful in assessing the physical properties of the estrogen receptor. A characteristic 8S molecular form of the cytoplasmic estrogen-receptor complex has been observed in buffers of low ionic strength (Erdos, 1968) whereas in buffers of high ionic strength the 8S form undergoes a reversible transformation to a more slowly sedimenting 4S form (Jensen and DeSombre, 1973; Notides and Nielson, 1974). Stancel et al. (1973) suggested that the 4S form most likely represented the "native" form of the receptor with the 8S form apparently reflecting an aggregate of several protein subunits detectable after tissue homogenization. Since studies of the receptor can be conducted only after estrogen is bound, the characteristics of the receptor prior to binding of the hormone in the cell are unknown. However, Notides and Nielson (1974) forwarded the most complete analysis of the 4S estrogen receptor. They concluded that the receptor is a protein of 76,000 daltons and of an asymmetric shape. The asymmetrical shape of the receptor is believed to be due to conformational changes that are caused by varying experimental conditions such as buffer composition and pH.

The high affinity binding of estrogen to its cytoplasmic receptor is characterized by an equilibrium constant (K_d) of 10^{-9} to 10^{-10} M (Gorski et al., 1968), the absence of cooperativity, and an apparent single estrogen binding site per receptor molecule (Peck et al., 1973; Williams and Gorski, 1974). The estrogen-receptor complexes undergo subsequent translocation to the nucleus (Jensen et al., 1968; Gorski et al., 1968) as characterized by nuclear accumulation of bound estrogen with concomitant depletion of cytoplasmic receptors (Shyamala and Gorski, 1969; Giannapolous and Gorski, 1971).

Coincident with nuclear uptake of the estrogen-receptor complex, the sedimentation coefficient of the receptor increases from 4S to 5S (Jensen et al., 1969; Shyamala and Gorski, 1969; Giannapolous and Gorski, 1971). It is not known whether the cytoplasmic receptor is transformed before or as a result of translocation (Gorski and Gannon, 1976). Incubation of uterine cytosol with estradiol at 25 to 37C but not at 0C results in the 4S to 5S transformation (Jensen et al., 1971) and appears to require prior binding of estradiol. The 5S, but not the 4S form exhibits high affinity for uterine nuclei (DeSombre et al., 1972). The 5S form has an estimated molecular weight of 130,000 to 140,000 and may represent a dimer consisting of the 4S receptor and some other monomeric protein that may or may not be identical to the 4S form (Notides and Nielson, 1974). A current hypothesis proposed and substantiated by Notides (1978) suggests that the 4S receptor is in equilibrium in the cytoplasm with some macromolecular inhibitor. Subsequent estradiol binding favors dissociation of the inhibitor which allows dimerization of the 4S receptor to another monomer to form the 5S conformation which then enters the nucleus.

The estradiol- and temperature-dependent translocation process seems not to involve energy, microtubules, or RNA and protein synthesis (Gorski and Gannon, 1976). Rather, activation of the receptor by some allosteric interaction with estradiol may serve in the creation of a steroid-receptor complex gradient between the cytoplasm and nucleus, thereby "driving" them into the nuclear compartment (Gorski and Gannon, 1976).

Properties of the Progesterone Receptor

Most of the insight regarding the nature of the mechanisms by which progesterone exerts its influence on reproductive organs has been provided by O'Malley et al. (1969) using the chick oviduct as a model. Although the results of the unprecedented and elegant studies conducted by these researchers have not been yet entirely verified in uterine tissue per se, the proposed mechanism of action of progesterone has been virtually accepted as being true for all species.

Initial studies conducted by Sherman et al. (1970) demonstrated a tissue-specific, heat-labile, acidic protein that binds progesterone with high affinity ($K_d = 10^{-10}$ M). The protein sedimented in sucrose gradients at 8S in low ionic strength buffer and at 4S in high ionic strength buffer. The molecular weight of the 4S form was estimated at 90,000 daltons. Partially purified progesterone receptors obtained from oviduct cytosol revealed the presence of two separate binding components (Schrader and O'Malley, 1972) that were designated A and B. Both had sedimentation coefficients of 4S in the presence of high salt, but eluted at different positions from DEAE-cellulose columns. At

low salt concentrations, A aggregated to form an 8S component, while B did not aggregate and remained at 4S. Both subunits were identical with respect to their hormone binding specificity and kinetics. It was postulated that the two components might be subunits of a larger complex (O'Malley et al., 1972). Subsequently, a 6S form of the coupled components was detected (Schrader et al., 1975). The progesterone receptor B subunit has been purified to apparent homogeneity (Schrader et al., 1977) and characterized (Kuhn et al., 1977). The pure B subunit has been shown to consist of a single polypeptide chain of 115,000 daltons mainly composed of acidic amino acid residues, with a single amino-terminal lysine, and being prolate-ellipsoid in shape (Kuhn et al., 1977).

Upon exposure of the chick oviduct to progesterone, an increase in extractable nuclear receptor occurred with a simultaneous decrease in the amount of cytoplasmic receptor (O'Malley et al., 1972). These data suggested that the progesterone-receptor complex, like the estrogen-receptor complex, was translocated to the nucleus. However, the mechanism of the translocation process for the progesterone-receptor complex, like the estrogen-receptor complex, has not been elucidated.

As might be expected, the sequela to the discovery of the uterine estrogen receptor was the demonstration of a soluble protein receptor that bound progesterone with high affinity ($K_d = 10^{-9}$ M) in the uterine cytosol of a number of mammalian species. The presence of this receptor proved more difficult to elucidate as the steroid-receptor complex was heat-labile, dissociated at a relatively rapid

rate (Feil et al., 1972), and was not always distinguishable from a corticosteroid binding globulin (CBG)-like protein that was also present in the uterus (Milgrom and Baulieu, 1970; Baulieu et al., 1975). Previous measurements of nuclear progesterone receptors in mammalian uteri were based upon extraction with KCl (Feil and Bardin, 1975) which, Clark and Peck (1976) suggest, excludes significant portions of the nuclear population of receptors. The availability of a synthetic progestin, 17,21-dimethyl-19-nor-4,9-pregnadiene-3,20-dione (R5020), has greatly facilitated the validation of assays for progesterone receptors (Philibert and Raynaud, 1973). This progestin was demontrated to bind with high affinity to the receptor, form a more stable slowly dissociating complex than progesterone, and not bind with any affinity to CBG-like proteins (Philibert and Raynaud, 1974). The biological activity and biochemical characteristics of R5020 have been reviewed by Raynaud (1977).

The uterine progesterone receptor present in the cytosol has been shown to sediment on sucrose gradients as a 7S form in the absence of salt and as a 4S form in a gradient containing 0.3-0.4 M KCl (Corvol et al., 1972). Analogous sedimentation properties were obtained by Kontula (1975) in ovine endometrial and myometrial cytosol preparations.

Influence of Steroid Hormone-Receptor Complexes on the Target Cell Genome

Since the central theme of the mechanism of action of steroid hormones seemed to involve the nucleus, the steroid hormone itself or some other intracellular mediator was presumed to bind to certain

predetermined sites in the nucleus. It was hypothesized that alterations in DNA transcription would occur leading to changes in nuclear RNA synthesis. Specific changes in gene expression would be reflected by increased synthesis of specific mRNAs' that should be limited to the target tissue and inducible only by the steroid in question. The basis for the "nuclear acceptor hypothesis" was first demonstrated by O'Malley et al. (1971) who showed that the progesterone-receptor complex could only bind with high affinity to nuclei of the oviduct cells but not to nuclei of nontarget cells. In this same study, fractionation of nuclei exposed to labeled progesterone receptor-complexes showed attachment of the complexes to the chromatin. In an attempt to identify which fraction of chromatin was responsible for the binding, histone proteins were selectively dissociated from chick oviduct chromatin and the remaining chromatin was reconstituted by dialysis (O'Malley et al., 1972; Spelsberg et al., 1971). Binding of the complexes to reconstituted chromatin was similar to the intact native chick oviduct chromatin. "Hybrid" chromatin containing histones from other tissues or species retained the capacity to bind progesterone-receptor complexes. Oviduct chromatin lacking all histones still showed more extensive binding than spleen chromatin minus histones. It was therefore concluded that histones were not primarily responsible for specificity of receptor binding. Indeed, when non-histone (acidic) proteins were removed, the chromatin of the chick oviduct lost most of its capacity to bind complexes (Spelsberg et al., 1971). When non-histones of chick erythrocytes were inserted into oviduct DNA during reconstitution, the ability to bind receptor was lost;

conversely, when non-histones from oviduct were inserted into erythrocyte chromatin, the binding capacity resembled that of native oviduct chromatin (Spelsberg et al., 1972). The localization of acceptor capacity has been demonstrated in the AP_3 non-histone fraction of target cell nuclei (Spelsberg et al., 1972). The B subunit of the progesterone receptor has been shown to bind to the non-histone protein DNA complexes, while the A subunit binds to pure DNA, but poorly to chromatin (O'Malley et al., 1973). The B subunit has been designated the specifier and the A subunit has been suggested to be the actual gene regulatory protein (O'Malley et al., 1976). Subsequent experiments proved that the B subunit alone did not stimulate RNA initiation sites on the chromatin as were observed with intact A-B dimers (Schrader et al., 1977). Schrader and O'Malley (1978) have recently demonstrated that the B subunit of the progesterone receptor dimer binds to the AP_3 non-histone regions of the chromatin allowing the subsequent dissociation of the A subunit to bind to DNA. These investigators suggested that the A subunit may act as an unwinding protein, destabilizing the DNA sufficiently so that RNA polymerase-DNA complexes can be formed while the non-histones may serve as "flags" in the genome to attract the receptor-complexes to the proper gene for activation and expression.

The nuclear acceptor hypothesis for the estradiol-receptor complex has not been described as precisely as for the progesterone-receptor complex and remains a controversial subject in the mechanism of action of this steroid. Using various assay systems, a number of studies identified different nuclear components as the specific acceptor sites.

Many earlier investigators had shown estrogen-receptor complexes associated with crude nuclear chromatin (King et al., 1966; Mauer and Chalkley, 1967; Toft, 1972). The results of other studies demonstrated that these complexes bind directly to DNA (Harris, 1971; King and Gordon, 1972). Jackson and Chalkley (1974) implicated nuclear membranes as acceptor sites. Utilization of affinity chromatography also showed that acceptors were located in non-histone protein fractions (Puca et al., 1974). Senior and Frankel (1978) exquisitely demonstrated that estradiol-receptor complexes from rat uteri bound to nucleosomes in the DNA strands and the linker region between them. Many of the above researchers concluded that the acceptor binding was of high affinity and displayed features of a saturable system. The validity of these experiments was challenged by Chamness et al. (1974) who demonstrated that the apparent saturation of nuclear acceptor sites was due to other cytoplasmic proteins that compete for the acceptors in the nucleus. By keeping the total protein content of the system constant while increasing the concentration of estrogen-receptor complexes added to nuclei, no saturation was observed (Chamness et al., 1973, 1974). However, Buller et al. (1975) utilizing an experimental design patterned after that of Chamness, demonstrated that saturation of chick oviduct acceptors did occur, and concluded that complexes have high affinity for a limited number of binding sites. Data from Clark et al. (1976) have shown the presence of two different binding sites of the estrogen-receptor complex in the nucleus: one form tightly bound to chromatin that is resistant to KCl extraction, and another more loosely bound to chromatin that can be extracted with KCL. These two forms showed temporal relationships suggesting that the resistant forms represent those complexes which are

specifically bound and are involved in mediating transcriptional events leading to true uterine growth.

It appears that the vast majority of steroid-receptor complexes might be redundant if only a few complexes are needed to provoke the full biological response. Thus, observed responses to hormone treatment would be insensitive to the degree of receptor saturation with hormone. However, dose-response relationships have demonstrated the response to be roughly proportional to the amount of complexes formed (Katzenellenbogen and Gorski, 1972; Tomkins, 1970), so that half-maximal response is obtained when only 3000 to 7000 receptor molecules per cell have entered the nucleus. Yamomoto and Alberts (1975) stated that the above data did not rule out the possibility that a hormone-receptor complex recognized only one specific DNA sequence and postulated that a specific response resulted from the location of only a few of these acceptor sites in an environment that permits them to be functional. These researchers proposed that a small number of high affinity acceptor sites that lead to a response do exist, but their presence is masked by the much larger number of low affinity sites that are without biological effect.

Although the precise nature of nuclear acceptor sites remains obscure, the accumulation of data implicating that steroids do indeed act at the genome cannot be ignored. Research conducted with genetic mutants containing alterations in steroid receptors could contribute valuable information about the nature of the interaction of the steroid with the genome (Yamomoto et al., 1976). The recent production of an antibody against the estrogen-receptor complex may aid in

the elucidation of acceptor site specificity (Greene et al., 1977). Finally, the determination of the ovalbumin gene sequence which is directly acted upon by estrogen may lead to even greater insight regarding the nature of the acceptor site (Lai et al., 1978).

Initiation of Subsequent Nuclear Events

Estrogen stimulates DNA-dependent RNA synthesis in the uterus within the first 6 h following hormone administration (Hamilton, 1968; Knowler and Smellie, 1971). The earliest detectable increase in RNA synthesis reported by Means and Hamilton (1966) occurs within 2 min after estrogen treatment. Thirty minutes after estradiol administration, an increase in high molecular weight RNA (DNA-like) occurs which may be requisite for the increase in total RNA that follows (Knowler and Smellie, 1971; Luck and Hamilton, 1972; Borthwick and Smellie, 1975). The most marked stimulation is manifested as an increase in ribosomal RNA synthesis that occurs at 4 to 6 h post-treatment (Hamilton, 1968; Billing et al., 1968).

The initial observations of Roeder and Rutter (1969) established the existence of three chromatographically separable DNA-dependent RNA polymerases in eukaryotic nuclei later referred to as I, II, and III. A subsequent study revealed the localization of polymerase I within the nucleolus while the nucleoplasm contained primarily polymerases II and III (Roeder and Rutter, 1970). Blatti et al. (1970) demonstrated the role of RNA polymerase I in the transcription of ribosomal RNA. The function of polymerase II in mRNA transcription was established after it was shown that this enzyme was inhibited by

low concentrations (10^{-10} to 10^{-7} M) of a peptide toxin, α-amanitin (Lindell et al., 1970; Kedinger et al., 1970). Similarly, RNA polymerase III, also inhibited by α-amanitin but at much higher concentrations (up to 10^{-4} M), was demonstrated to catalyze the transcription of 4 and 5S RNA (Weinman and Roeder, 1974). Thus, a number of investigators suggested that transcription may be quantitatively and qualitatively influenced by the levels of polymerase activities. Subsequent research was conducted to determine whether qualitative changes in RNA synthesis during various estrogen-induced functional transitions of the uterus were accompanied by changes in polymerase activities.

Coincident with the early temporal relationship between estrogen administration and uterine RNA synthesis an increase in RNA polymerase II activity 30 to 60 min post-treatment has been observed (Glasser et al., 1972; Borthwick and Smellie, 1975; Hardin et al., 1976). Approximately 4 to 6 h later an increase in RNA polymerase I activity and a second increase in the activity of RNA polymerase II has been demonstrated (Glasser et al., 1972; Borthwick and Smellie, 1975; Hardin et al., 1976). Administration of α-amanitin (Glasser et al., 1972) and actinomycin D (Means and Hamilton, 1966; Glasser and Spelsberg, 1973), but not cyclohexamide (Glasser and Spelsberg, 1973) to rats prior to estradiol treatment has been shown to block the initial increase in RNA polymerase II activity. All three inhibitors were able to suppress the second increase in RNA polymerase II activity and the stimulation of polymerase I activity induced by estradiol (Glasser and Spelsberg, 1973). It has been suggested that this early increase in RNA polymerase II activity and presumptive mRNA synthesis

may be involved in subsequent uterine protein synthesis which in turn is associated with the later increases of both polymerase activities and other uterine responses (Knowler and Smellie, 1971; Glasser et al., 1972; Raynaud-Jammet et al., 1972; Borthwick and Smellie, 1975).

Estradiol-induced stimulation of chromatin template capacity, which permits an estimate of the percentage of the total genome that is available for transcription by the endogenous RNA polymerases, has been reported (Teng and Hamilton, 1969; Glasser et al., 1972). A modification of this template assay enabled investigators to demonstrate that estrogen provokes a marked increase in the number of initiation sites for RNA polymerase binding in chick oviduct chromatin (Tsai et al., 1975; Kalimi et al., 1976).

It seems reasonable that an increased number of initiation sites for RNA polymerase binding under estrogen influence should lead to transcription of a specific mRNA and the subsequent translation of a specific protein induced by estrogen. This contention has been investigated by monitoring the appearance of ovalbumin, a protein present in large amounts in the estrogen-primed chick oviduct (O'Malley et al., 1969). By verifying the existence of ovalbumin mRNA following estrogen administration in vivo and in a cell-free system, these elegant studies provided evidence that estrogen indeed affects a change in gene expression (O'Malley and Means, 1974). Although the existence of a specific mRNA inducible by either estrogen or progesterone in the uterus remains to be elucidated, indirect evidence has indicated an estrogen-stimulated increase in uterine synthesis of mRNA for glucose-6-dehydrogenase (Smith and Barker, 1974).

Uterine DNA synthesis and cell proliferation has been shown to occur 18 to 30 h after estrogen administration (Epifanova, 1966; Kaye et al., 1972). Recently, Harris and Gorski (1978) demonstrated a corollary increase in DNA-dependent DNA polymerase, the enzyme required for replication, in uteri of immature rats treated with estradiol. This observation suggests a role for this enzyme in DNA replication, the ultimate event in the sequence of estradiol-induced responses in the uterus.

The effects of progesterone on uterine RNA and protein synthesis are less dramatic than those observed after estrogen treatment. Studies conducted on nuclear events elicited by progesterone in the uterus are relatively few in number. Progesterone administered to ovariectomized estradiol-primed rats has been demonstrated to inhibit uterine RNA and protein synthesis (Trams et al., 1973). Transient increases in uterine RNA polymerase I and II activities at 15 and 30 min, respectively, after an injection of progesterone into estradiol-primed rabbits were followed by a rapid decline and a subsequent overall depression of both enzyme activities suggesting an antagonistic effect of progesterone (Kokko et al., 1977). In contrast, Stone et al. (1978) reported that progesterone administered sequentially for 3, 6, or 9 days prior to a 3 day sequence of estradiol, as well as an estradiol plus progesterone treatment for 3 days was unable to suppress the stimulatory effects of the estrogen alone on uterine RNA and protein synthesis in ovariectomized ewes.

Administration of progesterone alone caused no marked changes in total cellular RNA synthesis in the chick oviduct (Means and O'Malley,

1971). However, progesterone has been shown to specifically control oviductal synthesis of the egg-white protein, avidin (O'Malley et al., 1969). Although the concentration of avidin in the total egg-white protein is small, a specific mRNA for this protein has been isolated following progesterone treatment (Chan et al., 1973) with maximum activity observed by 18 h post-treatment. In estrogen-treated chicks withdrawn from hormonal treatment for 12 days, a subsequent single injection of progesterone resulted in a rapid increase in the number of oviduct chromatin initiation sites that appear to be similar sites enhanced by estrogen (Schwartz et al., 1977). In addition, ovalbumin mRNA accumulated within 4 h after progesterone treatment of withdrawn chicks and continued to increase by 24 h (Schwartz et al., 1977). Treatment of withdrawn chicks with progesterone caused a decrease in RNA polymerase I and II activities as well as chromatin template capacity, while estrogen plus progesterone enhanced both polymerase activities (Spelsberg and Cox, 1976). Interestingly, progesterone demonstrated no antagonism with respect to ovalbumin synthesis in this system suggesting that the antagonism between the two hormones does exist at a transcriptional level but may not specifically affect transcription of the gene sequences coding for ovalbumin (Spelsberg and Cox, 1976). These data aided in the verification of the mechanism by which steroids effect nuclear changes in target cells that result in the synthesis of specific mRNAs necessary for a biological response.

Regulation of Uterine Steroid Hormone Receptor Concentrations

Modification of steroid hormone action of the uterus appears to be dictated by the concentration of receptors. The nature of the regulatory mechanism has been investigated experimentally by modulating steroid hormone levels in ovariectomized and immature animals and by correlating endogenous fluctuations in hormone with receptor levels during the estrous or menstrual cycle. Changes in the quantity and compartmentalization of estrogen and progesterone receptors under the above conditions have been observed mainly in the uteri of laboratory animals. Recently, these observations have been extended to include domestic animals.

Control of Estrogen Receptor Concentrations

Uterine estrogen receptor concentrations in the cytoplasm and nucleus are principally affected by two major classes of hormones: estrogens and progestins. In addition, certain exogenous non-steroidal compounds termed "antiestrogens" have been shown to affect cytoplasmic and nuclear concentrations of the estrogen receptor.

Considerable evidence exists that estrogens stimulate uterine estrogen receptor synthesis. This aspect of estrogen action has been extensively studied in the immature or ovariectomized rat. Subsequent to one injection of estradiol, the increase in the quantity of nuclear estrogen receptors is dose-dependent with maximal levels attained at 1 h after treatment (Anderson *et al.*, 1973). The decline in nuclear estrogen receptors is also dose-dependent, but equivalent quantities

at all doses are observed at 6 h with a return to control levels by 24 h post-injection (Anderson et al., 1973). Estradiol administration 24 h after an initial injection of hormone induces a greater quantity of nuclear estrogen receptors than the maximum observed 1 h after the first injection (Anderson et al., 1974).

After the initial depletion of cytoplasmic receptors following estradiol administration, two separate phases may be involved in receptor replenishment (Hsueh et al., 1976). The first phase, 4 to 8 h post-injection, appeared to represent recycling of the receptors because the quantity depleted is equal to that replenished (Hsueh et al., 1976). The second phase, 8 to 24 h after injection, involved synthesis of new receptors since the quantity of receptor at 24 h was greater than that initially present (Hsueh et al., 1976; Bhakoo and Katzenellenbogen, 1977). Sarff and Gorski (1971) utilizing cyclohexamide, an inhibitor of protein synthesis, suggested that receptor synthesis occurred during the recycling phase. However, Mester and Baulieu (1975) observed that the first phase was insensitive to cyclohexamide inhibition, whereas the latter phase appeared to be dependent upon protein synthesis.

Progesterone has a negative effect on uterine estrogen receptor concentrations. Administration of progesterone to estradiol-primed immature rats (Hsueh et al., 1976; Bhakoo and Katzenellenbogen, 1977), ovariectomized cats (West et al., 1976) and ewes (Koligian and Stormshak, 1977a) reduced uterine binding of estrogen due to an inhibitory effect on the quantity of available cytoplasmic estrogen receptors. This antagonism by progesterone was observed to occur during

the second or synthetic phase of receptor replenishment and did not appear to result from changes in steroid binding specificity or the binding affinity of the cytoplasmic estrogen receptor (Hsueh et al., 1976). Progesterone was also without an inhibitory effect on the translocation of estrogen-receptor complexes to the nucleus (Hsueh et al., 1976), but reduced the nuclear retention time of the estrogen receptor complexes. The ability of progesterone to reduce the nuclear retention time of the estrogen-receptor complex may contribute to the overall mechanism of its antagonism.

Estrogen receptor concentrations may also undergo regulation by other steroidal and non-steroidal estrogens. Estriol, a short-acting estrogen, stimulated all early uterotropic events, but failed to induce late responses due to a rapid loss of the estriol-receptor complexes from the nucleus (Anderson et al., 1975). A single injection of estriol increased cytoplasmic receptor levels by 24 h (Clark et al., 1977). In this latter study, cytoplasmic receptor levels were also increased by 48 h after the first of two sequential injections of estriol. However, simultaneous administration of estriol in combination with estradiol antagonized the stimulatory effect of estradiol on cytoplasmic receptor levels (Clark et al., 1977). Since estriol was capable of increasing cytoplasmic receptors, lack of available receptors was not likely the source of antagonism. Rather, the nature of this antagonism appeared to involve competition between estradiol- and estriol-receptor complexes for nuclear retention sites, thus reducing the number of estradiol-receptor complexes necessary to elicit a greater increase in cytoplasmic receptor levels (Clark et al., 1977).

Nuclear binding of the estrogen receptor induced by the non-steroidal antiestrogens, nafoxidine or clomiphene, resulted in retention of the complexes for very long periods of time and thus, stimulation of both early and late uterotropic events (Clark et al., 1973, 1974). However, these and other antiestrogenic compounds failed to stimulate cytoplasmic receptor replenishment (Clark et al., 1973, 1974; Ferguson and Katzenellenbogen, 1977) that lasted up to 72 h (Clark et al., 1978). This depletion of cytoplasmic receptor sites by antiestrogens may underlie the observed inability of uterine cells to respond to subsequent estradiol administration with increases in cytoplasmic receptors or continued uterine growth (Clark et al., 1973, 1974; Ferguson and Katzenellenbogen, 1977).

Control of Progesterone Receptor Concentrations

Results from a number of investigations have shown that the concentration of uterine progesterone receptors is under dual steroidal control.

Estradiol-17β is a positive regulator of cytoplasmic progesterone receptor synthesis. Administration of this estrogen to ovariectomized rats (Milgrom and Baulieu, 1970; Feil et al., 1972), rabbits (Faber et al., 1973; Rao et al., 1973), mice (Feil et al., 1972), hamsters (Leavitt and Blaha, 1972), and guinea pigs (Corvol et al., 1972; Freifield et al., 1974) increased progesterone receptor concentrations, thus enhancing the ability of the uterus to respond to progesterone. The induction of new receptors apparently reflected de novo synthesis since estradiol-17β treatment 14 days after ovariectomy

restored the concentration of progesterone receptors to levels observed at proestrus by 24 h in the hamster (Leavitt et al., 1974). This effect of estradiol was abolished when actinomycin D or cyclohexamide were administered 15 min before hormone injection, indicating a dependence of receptor synthesis on RNA and protein synthesis, respectively (Milgrom et al., 1973). When these inhibitors were injected 20 h or more after estradiol, progesterone receptor levels were maintained at their stimulated level (Vu Hai et al., 1977). Similarly, uterine strips treated with estrogen in vitro and subsequently exposed to actinomycin D or cyclohexamide at 6 h of incubation retained elevated progesterone receptor concentrations measured at 12 h (Leavitt et al., 1977). These authors suggested that estrogen stimuation of RNA synthesis during the first 6 h was adequate to maintain progesterone receptor synthesis in the absence of new RNA from 6 to 12 hours.

Recently, a number of antiestrogenic compounds (nafoxidine, clomiphene, CI-628) have been shown to be capable of increasing uterine progesterone receptor levels, albeit to a lesser extent than estradiol-17β (Leavitt et al., 1977). Since these agents were unable to increase estrogen receptor replenishment as much as estradiol, it has been postulated that antiestrogens preferentially increase progesterone receptor levels, thus rendering the uterus more responsive to progesterone than to estrogen (Leavitt et al., 1977).

In contrast to the effects of estrogen, progesterone seems to decrease the concentration of its own receptor. Experiments conducted by Milgrom et al. (1973) indicated that progesterone administration

depleted cytoplasmic receptors within a few hours which lasted for several days. This decrease exceeded the amount of receptor translocated to the uterine nuclei, could not be attributed to an inhibition of receptor synthesis, and did not seem to require protein synthesis (Leavitt et al., 1977). It is not known whether this inactivation of the progesterone receptor was due to degradation of the receptor protein itself or modification of ligand-receptor binding. A similar phenomenon has been recently observed in progesterone-treated rabbit uteri where depletion of the cytosol receptor persisted even after nuclear receptor levels had returned to control values (Janne et al., 1978). Thus, it has been suggested that there is an autoregulation of progesterone action, because as the hormone affects the uterine cell it decreases the concentration of its own cytoplasmic receptor.

Cyclic Changes in Uterine Steroid Receptor Concentrations

The variations in uterine steroid hormone receptor concentrations during the estrous cycles of many laboratory animals have been reviewed (Brenner and West, 1975). Briefly, the levels of cytoplasmic steroid receptors are highest during proestrus, which is correlated with maximal ovarian estrogen secretion, and lowest during metestrus. These fluctuations in receptor concentrations can be explained by the regulatory effects of estrogen since many of these animals lack any distinct luteal phase with increased secretion of progesterone.

Subsequent studies were conducted utilizing species with comparatively longer estrous or menstrual cycles and produced similar results.

The period of proestrus to early metestrus is characterized by increased uterine concentrations of cytoplasmic estrogen receptors in the cow (Senior, 1975; Henricks and Harris, 1978), ewe (Koligian and Stormshak, 1977b; Miller et al., 1977) and gilt (Pack et al., 1978). Similar increases in cytoplasmic estrogen receptor concentrations occur prior to and immediately after ovulation in the woman (Bayard et al., 1978). Concentrations of nuclear bound estrogen are also increased during and slightly after these stages, suggesting translocation of the steroid-receptor complexes to this cellular compartment (Koligian and Stormshak, 1977b; Pack et al., 1978; Bayard et al., 1978). These increases in cytoplasmic and nuclear bound steroid are consistent with the increased levels of estrogen in the systemic blood during these stages. Due to the increased secretion of estrogen and its effect on receptor synthesis, endometrial cytoplasmic progesterone receptor concentrations are also increased during these stages of the cycle in the ewe (Miller et al., 1977) and woman (Bayard et al., 1978). Nuclear bound progesterone during proestrus, estrus, and metestrus is low due to the lack of availability of sufficient ovarian progesterone to bind to the cytoplasmic receptors (Bayard et al., 1978).

As the cycle progresses to the luteal or secretory phase, cytoplasmic receptor levels of both steroids have been found to decrease in all species. The corollary increase in circulating progesterone levels has been shown to antagonize the replenishment of cytoplasmic estrogen receptors (Koligian and Stormshak, 1977a). Tseng and Gurpide (1975) demonstrated the induction of estradiol dehydrogenase

by progestins in the human endometrium. It has been suggested that the presence of this estradiol-metabolizing enzyme decreased the availability of estradiol which may account for the observed decrease in cytoplasmic and nuclear estrogen receptors. Analogous to these results, the appearance of estrogen sulfotransferase in the gilt endometrium during the luteal phase may account for the observed reduction in nuclear bound estrogen (Pack and Brooks, 1974; Pack et al., 1978). The decline in cytoplasmic progesterone receptor levels during this phase has been explained by the ability of this hormone to inactivate its own receptors as described by Milgrom et al. (1973). This remains to be demonstrated in uteri of domestic animals. In addition, the decrease in plasma estradiol and estrogen receptors may lead to decreased inductive effects of this steroid on cytoplasmic progesterone receptor levels. Nuclear progesterone receptors were increased at this time (Bayard et al., 1978), possibly reflecting the translocation process. The integration of circulating ovarian steroids and their respective receptors thus leads to modulation of uterine function throughout the cycle.

Uterine Regulation of Luteal Function in the Ewe

Local Luteolytic Effect of the Uterus

The demonstration that hysterectomy prolonged corpus luteum function in the ewe led to the implication that the uterus plays a major role in the regulation of luteal lifespan in this species (Wiltbank and Casida, 1956). A local luteolytic effect of the uterus appeared to exist since unilateral hysterectomy of ewes resulted in

regression of corpora lutea in ovaries ipsilateral to the intact horn while those on the contralateral side were maintained (Inskeep and Butcher, 1966). Various experiments employing autotransplantation of reproductive tracts to the necks of ewes further verified the presence of a local effect of the uterus in regulating the lifespan of the corpus luteum (McCracken et al., 1971). Subsequent to the suggestion by Pharris and Wyngarden (1969) that prostaglandin $F_2\alpha$ ($PGF_2\alpha$), because of its relative abundance in the uterus, may be the uterine luteolysin, McCracken et al. (1972) proved that $PGF_2\alpha$ was indeed the luteolysin in the ewe. Uterine venous blood from a donor ewe on day 15 of the cycle infused into the arterial supply of the transplanted ovary of a recipient ewe induced luteal regression similar to that seen with intra-arterial infusion of $PGF_2\alpha$ (McCracken et al., 1972).

Separation of the ovarian artery from the utero-ovarian vein prevented regression of the corpus luteum in the ewe suggesting that the luteolysin in uterine venous blood reached the ovary via a counter-current mechanism between this vein and the adherent ovarian artery (Barrett et al., 1971). McCracken et al. (1972) confirmed this route of transport by demonstrating that infusion of tritium-labeled $PGF_2\alpha$ into the uterine vein resulted in a greater amount of radioactivity present in ovarian arterial than in iliac arterial blood. Anastomoses of uterine veins or arteries in unilaterally hysterectomized ewes showed that uterine-induced luteal regression is exerted through a local veno-arterial pathway between a uterine horn and its adjacent ovary (Ginther et al., 1973). Anatomical studies beautifully demonstrated areas of close apposition between the uterine vein and the

tortuous tightly-coiled ovarian artery (Ginther and Del Campo, 1973) where the direct passage of $PGF_2\alpha$ most likely occurred. A subsequent study utilizing various surgical anastomoses confirmed the adequacy of the main uterine vein as an "outlet" for the luteolysin and the ovarian artery as the final component of the pathway from uterus to ovary (Mapletoft and Ginther, 1975). The precise nature of the transfer of the luteolysin from one vessel to the other is not fully understood, but perhaps involves diffusion through the intercellular spaces of the intervening vessel walls (Ginther, 1974).

Estrogen-Induced Luteal Regression

Administration of estradiol on either Day 11 and/or 12 of the estrous cycle induces premature luteal regression in intact, but not hysterectomized ewes (Stormshak et al., 1969; Hawk and Bolt, 1970). These results suggest that estradiol-induced luteal regression during the mid-luteal phase of the ovine estrous cycle is mediated by an effect of the hormone on the uterus. Exogenous estradiol is also capable of stimulating an increase in the release of $PGF_2\alpha$ from the ovine endometrium (Barcikowski et al., 1974). The exposure of the uterus to relatively high concentrations of endogenous progesterone during this phase of the cycle appears to be requisite for the luteolytic action of this estrogen (Warren et al., 1973; Barcikowski et al., 1974). Progesterone probably provokes intracellular changes that are essential for the uterus to respond to estrogen with increased synthesis and secretion of luteolysin. The estradiol-induced release of $PGF_2\alpha$ from the endometrium may represent de novo synthesis since this

response is blocked by indomethacin, an inhibitor of prostaglandin synthesis (Barcikowski et al., 1974).

The ability of exogenous estrogen to provoke luteal regression appears to be more dependent upon the duration of exposure of the uterus to the hormone than the dosage (Bolt and Hawk, 1972). This is supported by the observations of Chakraborty and Stormshak (1976) who determined that hysterectomy of ewes 24 h after a single injection of estradiol on Day 10 prevents regression of the corpus luteum, whereas hysterectomy 48 h after the first of two sequential injections induces luteolysis. Also congruent with these data is the demonstration by Ford et al. (1975) that treatment of ewes with two sequential injections of estradiol during the mid-luteal phase of the cycle provokes an increase in uterine synthesis and secretion of prostaglandins F at 18 h after the last injection.

Recalling the mechanism by which estrogen acts in the uterus to elicit its characteristic biological responses and the hormonal regulation of steroid receptors leads one to question how this steroid can induce luteal regression within a particular post-treatment period during a stage of the cycle when endogenous progesterone is maximal and concentrations of estrogen and progesterone receptors are minimal. The desire to investigate this apparent paradox provided the impetus for the following studies.

STATEMENT OF THE PROBLEM

The stimulatory action of estrogen on target tissue proliferation has long been recognized. Natural and synthetic estrogens, in particular diethylstilbestrol (DES), have been used extensively by the livestock industry as growth stimulants. The use of DES in finishing rations or as implants has been based upon the somewhat unique ability of this hormone to evoke general somatic growth. Estrogens have also been used as therapeutic agents in potentiating estrous behavior of ewes and cows and as abortifacients in these species.

Recently, considerable attention has been focused on the relationship between estrogens and the cellular proliferation of tumors found in the female reproductive tract and breast. Reports of a significant incidence of vaginal adenocarcinoma in daughters of women who had received DES treatment for the prevention of miscarriage have associated estrogen with carcinogenesis. The quantification of estrogen and progesterone receptors in actively proliferating human mammary tumors in conjunction with observations that 30% of these tumors regressed in response to endocrine ablation, suggested a principle role of estrogen in the stimulation of tumor growth. In addition, a significant proportion of menopausal women, those with estrogen-secreting ovarian tumors and those receiving exogenous estrogens have developed endometrial carcinoma. The measurement of steroid hormone receptors in this type of neoplastic tissue has also identified those tumors which are dependent upon estrogen for their growth.

The use of DES in the rations of food-producing animals is no longer tolerated in view of recent federal legislation prohibiting the

manufacture and use of this putative carcinogen in cattle and sheep. Knowledge of the biochemical mechanism(s) by which estrogen elicits its characteristic proliferative responses may ultimately aid in the elucidation of replacement substances that possess growth-promoting abilities without carcinogenic effects.

Basic knowledge concerning the cellular functions associated with the reproductive processes of domestic animals is limited. Research conducted at the cellular level may aid in understanding the role of steroid hormones in regulating target organ function during various reproductive states. The experiments which follow were conducted to determine the biochemical events associated with the mechanism whereby estradiol affects changes in uterine function in the ewe. The phenomenon of premature luteal regression induced by estradiol during the mid-luteal phase of the ovine estrous cycle was utilized for these studies because it provides a unique system with which to relate the mechanism of action of steroid hormones in the uterus with a distinct biological response.

EXPERIMENT I: INFLUENCE OF EXOGENOUS ESTRADIOL ON ENDOMETRIAL PROGESTERONE RECEPTORS DURING THE MID-LUTEAL PHASE OF THE OVINE ESTROUS CYCLE

Introduction

Research conducted approximately a decade ago demonstrated that estrogen-induced luteal regression during the mid-luteal phase of the estrous cycle of the ewe is mediated by an effect of the hormone on the uterus (Stormshak et al., 1969; Hawk and Bolt, 1970). Results of subsequent studies established that exposure of the uterus to the increased concentrations of progesterone present during this phase of the cycle is necessary for exogenous estrogen to induce luteolysis (Warren et al., 1973; Barcikowski et al., 1974). The treatment regime most often employed consists of one injection of estrogen on each of two consecutive days between Days 9 and 13 of the cycle (Stormshak et al., 1969; Hawk and Bolt, 1970; Warren et al., 1973). Although daily dosages of estrogen equal to or in excess of 250 μg were effective in provoking luteal regression, this response appeared to be more dependent upon the duration of exposure of the uterus to the hormone than the dosage (Bolt and Hawk, 1972). This is supported by the observation that hysterectomy 24 but not 48 h after initial treatment with estradiol prevented induced regression of the corpus luteum (Chakraborty and Stormshak, 1976). In addition, uterine synthesis and release of prostaglandin $F_2\alpha$, the luteolysin in the ewe (McCracken et al., 1972), was markedly increased 42 h after initial treatment with estrogen (Ford et al., 1975).

It is generally accepted that steroids are bound to cytoplasmic receptors after which the steroid-receptor complex is translocated to the nucleus where the hormone affects a change in target cell gene expression. Estrogen stimulates increased synthesis of progesterone receptors (Leavitt et al., 1974; Milgrom et al., 1973). Progesterone, however, appears to autoregulate concentrations of its own receptor either by degradation or an alteration of ligand-receptor binding (Milgrom et al., 1973). During the mid-luteal phase of the estrous cycle of the ewe progesterone secretion is near maximal and uterine concentrations of estrogen and progesterone receptors are minimal (Koligian and Stormshak, 1977b; Miller et al., 1977). Failure of exogenous estrogen to stimulate increases in receptor levels during the first 24 h after treatment may indirectly explain why further exposure of the uterus to this hormone is necessary for luteolysis to occur.

The present study was therefore conducted to monitor changes in the concentration of total cytoplasmic and nuclear progesterone receptors in the ovine endometrium at various intervals following estradiol administration. The incorporation of labeled thymidine into DNA was used as a corollary measure of the response of the uterus to estradiol.

Materials and Methods

Animals

Twenty mature crossbred ewes exhibiting normal estrous cycles of 16.3 $\pm$ 0.2 (SE) days duration, as determined by twice daily checks for

estrus with vasectomized rams, were utilized for this study (first day of detected estrus = Day 0).

Ewes were assigned randomly to 4 groups of equal numbers with each ewe receiving a single intramuscular injection of sesame oil (vehicle) or estradiol-17β (500 μg dissolved in 1.0 ml vehicle) on each of Days 11 and 12 of the estrous cycle (48 and 24 h prior to necropsy, respectively). The sequence of treatments for each group of ewes was as follows: Group 1, vehicle on Days 11 and 12; Group 2, vehicle on Day 11 and estradiol on Day 12; Group 3, estradiol on Day 11 and vehicle on Day 12; and Group 4, estradiol on Days 11 and 12. Immediately prior to necropsy, a blood sample was obtained by jugular venipuncture and the resulting serum frozen until quantified for progesterone using radioimmunoassay (Koligian and Stormshak, 1977b). All animals were sacrificed on Day 13 of the cycle.

Upon necropsy, uteri were excized, placed on ice, and transported to the laboratory within 20 minutes. Each uterine horn was split longitudinally and the endometrium was dissected from the myometrium. Further dissection was carried out in 0.15M NaCl to obtain a pool of intercaruncular endometrium. Tissues and buffer solutions were maintained at 0-4C unless otherwise noted.

Determiniation of Total Nuclear and Cytoplasmic Progesterone Receptors

Aliquots of intercaruncular endometrium from each ewe were subjected to progesterone receptor exchange assay as described by Clark and Peck (1977). Tissue was homogenized in an all-glass tissue grinder containing 1.0 ml of TEM buffer (10mM Tris-HCl, 1.5mM EDTA, 3.0mM

monothioglycerol, 10% glycerol, vol/vol; pH 7.4 at 25C). Homogenization consisted of 30 strokes with 30 sec intermittent cooling between each six strokes. Cytosol (supernatant) and a nuclear fraction (pellet) were obtained following centrifugation at 800 x g for 10 minutes. The supernatant was adjusted to a concentration equivalent to 100 mg endometrium/ml TEM buffer and centrifuged at 30,000 x g for 45 minutes. The resultant supernatant was readjusted to the same tissue concentration as above and subjected to exchange assay for cytoplasmic receptors.

The saturating concentration of [^{3}H]R5020 (17,21-dimethyl-19-nor-4,9-pregnadiene-3,20-dione) used for the exchange assay was determined by incubating duplicate 0.5 ml aliquots of endometrial cytosol (100 mg/ml) from three ewes in each group with 10 µl of increasing concentrations of [^{3}H]R5020 (83.0 Ci/mmol, New England Nuclear; 0.5, 1, 2, 4, 5, 8, 10 and 20nM in absolute ethanol), or with each concentration of labeled ligand plus a 100-fold excess of unlabeled R5020 (New England Nuclear). The saturating concentration of [^{3}H]R5020 was determined to be 1 x 10^{-8} M and was used in the incubation of subsequent samples. Exchange was conducted for 20 h at 4C. Tubes containing the supernatant were placed on ice, subjected to dextran-charcoal adsorption (0.5 ml, 0.05%:0.5% Norit A, Sigma, dissolved in 0.01M phosphate-buffered saline, pH 7.0, vol/vol, 10 min, vortexed at 5 min intervals) to remove any free ligand and centrifuged at 1500 x g for 10 minutes. A 0.5 ml aliquot of the supernatant was pipetted into a scintillation vial containing 10 ml scintillation fluid (0.7% 2,5-diphenyloxazole in toluene: Triton X-100, 2:1, vol/vol) and counted at 34% efficiency.

Concentration of nuclear progesterone receptors was determined using the pellet of nuclei obtained from the first centrifugation of endometrial homogenate. After resuspension of the pellet to a concentration equivalent to 100 mg endometrium/ml with TG buffer (10mM Tris-HCl, 30% glycerol, vol/vol; pH 7.4 at 25C), the pellet was washed three times with TG buffer. The same concentrations of [^{3}H]R5020 and labeled ligand plus R5020 used in the cytoplasmic exchange assay were added to duplicate 100 mg equivalent suspensions of nuclei and exchange was conducted at 4C for 20 hours. Following incubation, the sample was placed on ice and 1.5 ml TG buffer was added. Centrifugation for 10 min at 800 x g was followed by three additional washes of the pellet with 2.0 ml redistilled absolute ethanol for 16 h at 25C. Following extraction, the pellet was vortexed, centrifuged and the total supernatant was decanted into a scintillation vial for counting.

The difference between total binding (samples incubated with labeled R5020) and non-specific binding (samples incubated with labeled ligand and a 100-fold excess of R5020) represents the specific binding of steroid to the cytoplasmic and nuclear progesterone receptors. Specific binding of progestin to the cytoplasmic progesterone receptor averaged 75% of the total binding over all ligand concentrations used in the saturation analysis. Similarly, specific nuclear bound progestin represented 38% of the total binding.

Specificity of R5020 Binding

Endometrium, spleen, and diaphragm from each of the five control ewes (group 1) were subjected to exchange assay for cytoplasmic

progesterone receptors to determine tissue specificity for R5020 binding. The supernatant of each tissue homogenate was adjusted to 100 mg tissue/ml with TEM buffer and incubated in duplicate with 10nM [^{3}H]R5020 or [^{3}H]R5020 plus a 100-fold excess of unlabeled ligand.

In vitro Incorporation of [^{3}H]Thymidine into DNA and Determination of DNA

Endometrial samples of 58.2 ± 0.4 mg were incubated for 1 h at 39C in 2 ml Eagle's HeLa medium with 1 µCi of methyl-[^{3}H]thymidine (20 Ci/mmol, New England Nuclear). The samples were then homogenized in 2 ml 0.5M perchloric acid and stored at -20C for later determination of thymidine incorporation into DNA as described by Stormshak et al. (1976).

The DNA content of the endometrium from both uterine horns of all ewes and the spleen and diaphragm of control ewes was quantified by the method of Burton (1956).

Statistical Analysis of Data

Data were analyzed statistically by analysis of variance using single degree of freedom contrasts to determine the significance of differences between groups.

Results

Analysis of [^{3}H]R5020 Binding to Cytoplasmic and Nuclear Progesterone Receptors

Characteristics of the specific binding of [^{3}H]R5020 to progesterone receptors as a function of the final concentration of ligand in

endometrial cytosol of ewes receiving two sequential injections of estradiol (group 4) are presented in Figure 1. These data indicate that a concentration of 1 x 10^{-8}M labeled progestin saturates the progesterone receptors in cytosol from 100 mg endometrium/ml buffer. Specific binding of ligand to progesterone receptors in endometrial cytosol of ewes in the remaining three groups also indicated that a concentration of 1 x 10^{-8}M was saturating (data not shown). The data depicted in Figure 1 were subjected to analysis according to the methodology of Scatchard (1949), and are presented in Figure 2A. From this linear relationship (r^2 = .95), the equilibrium dissociation constant (K_d) for the progesterone-receptor complex was determined to be 1.85 nM with a concentration of cytoplasmic receptors of 1130 fmole/mg DNA. A concentration of 1 x 10^{-8}M [^{3}H]R5020 also saturated nuclear progesterone receptors of ewes in all four groups (data not shown). Scatchard analysis (r^2 = .85) of nuclear-bound labeled progestin from group 4 ewes revealed a K_d and concentration of nuclear receptors of .71 nM and 200 fmole/mg DNA, respectively (Figure 2B).

The specific binding of [^{3}H]R5020 to cytoplasmic receptors in the endometrium was approximately 10-fold greater than that determined in spleen (Table 1), and was non-detectable in diaphragm.

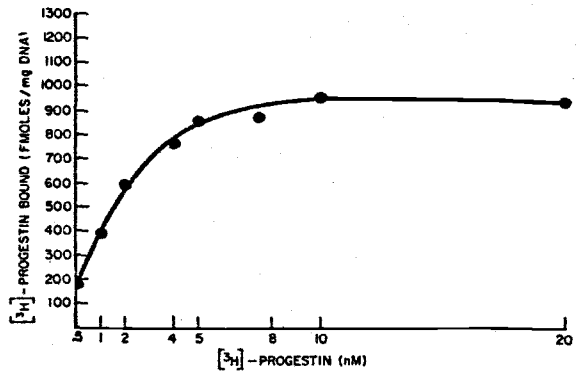


Figure 1

Specific binding of [^{3}H]R5020 to cytoplasmic progesterone receptors in the endometria of ewes receiving two sequential injections of estradiol. Samples of endometrial cytosol from each of three ewes were incubated in duplicate with each concentration of [^{3}H]R5020. Each point represents the mean of three ewes.

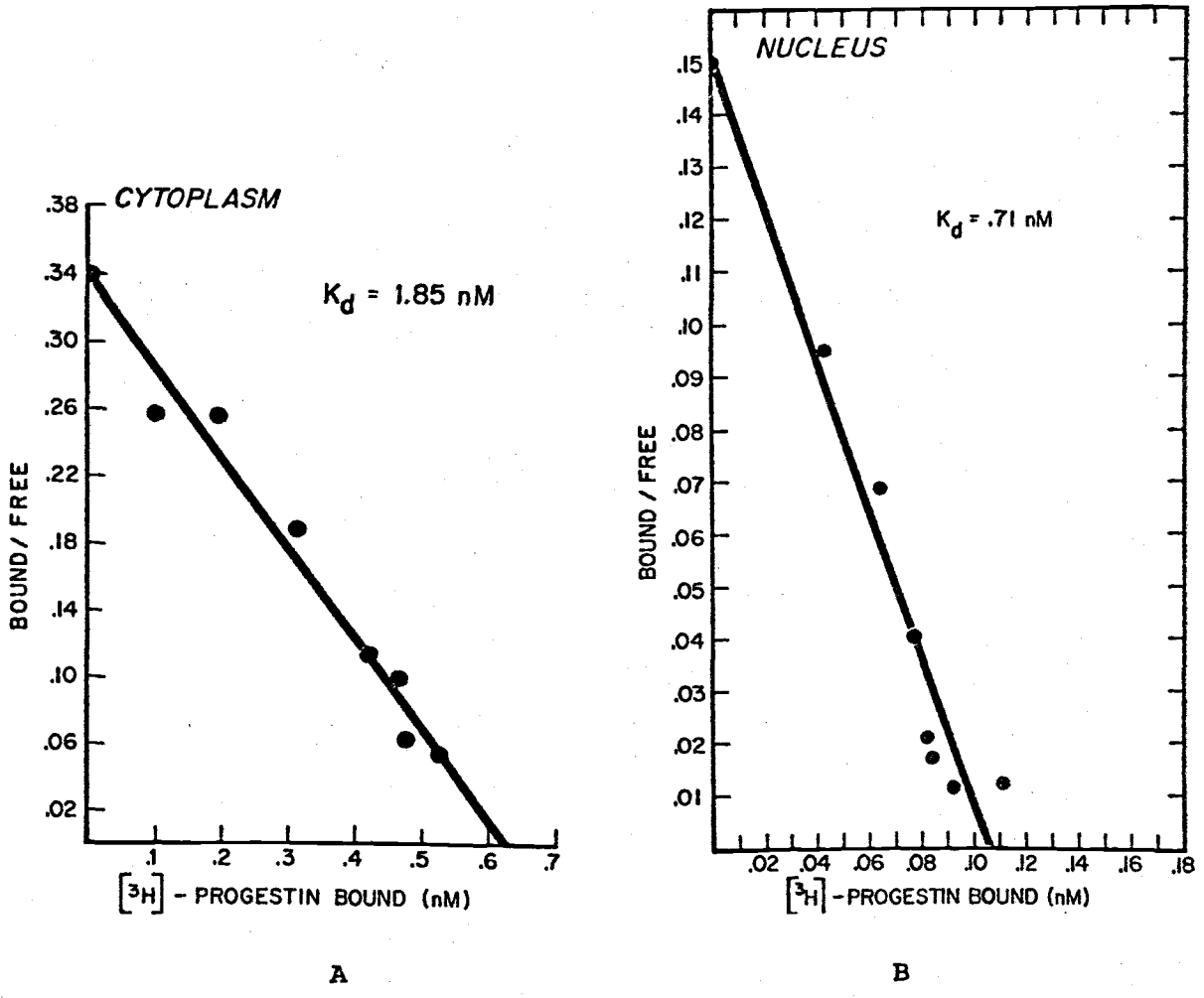


Figure 2

Scatchard analysis of specifically-bound [^{3}H]R5020 in endometrial cytosol (A) and nuclei (B) of ewes receiving two sequential injections of estradiol. Each point represents the mean of results of three ewes after exchange assay with various concentrations of [^{3}H]R5020. Bound/Free represents specifically bound [^{3}H]R5020 divided by unbound [^{3}H]R5020.

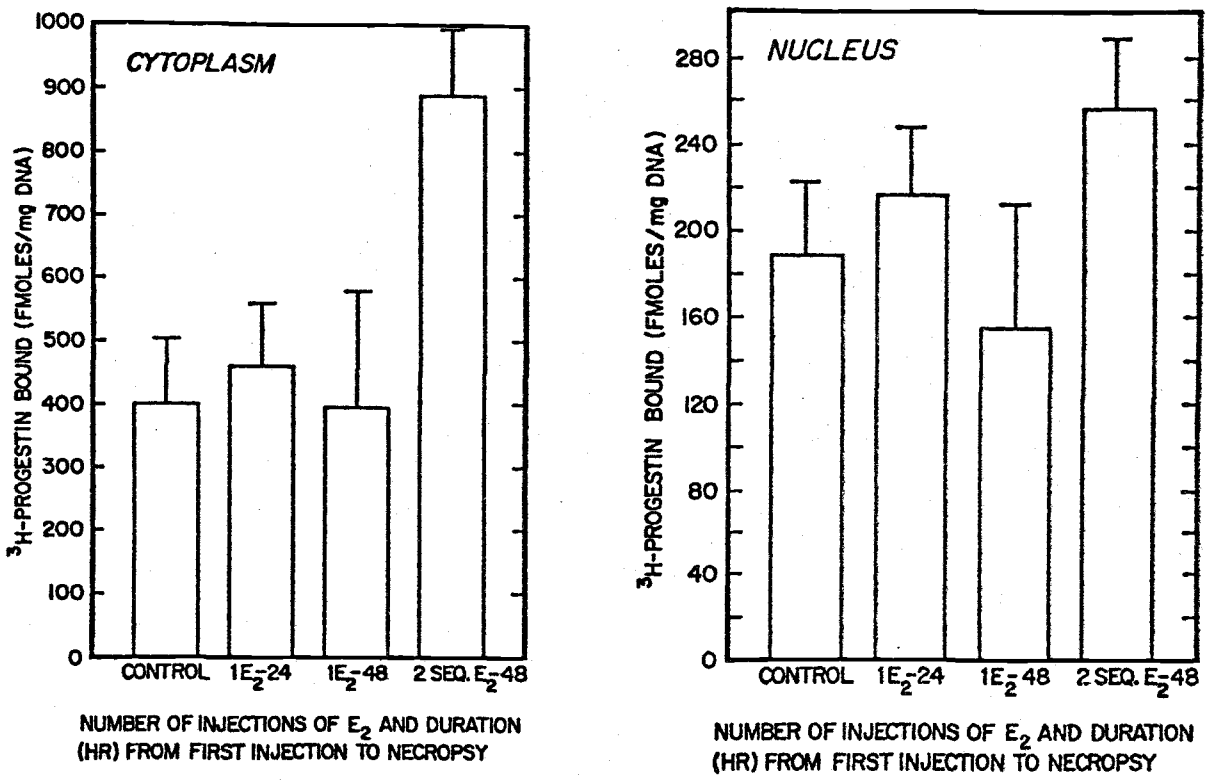
TABLE 1. Tissue specificity of cytoplasmic [^{3}H]progestin binding.

Tissue	Specific [^{3}H]R5020 Bound (fmoles/mg DNA)
Endometrium	401 ± 110[a]
Spleen	40 ± 5
Diaphragm	Not detected

[a]Mean ± SE of tissue from five ewes per group

Changes in Cytoplasmic and Nuclear Progesterone Receptors as a Result of Estradiol Treatment

Changes in the specific binding of [^{3}H]progestin to progesterone receptors in cytoplasm and nuclei of endometrium as determined by exchange assay are presented in Figures 3A and B. The concentration of cytoplasmic progesterone receptors in endometrium was greater ($P<.01$) in ewes receiving two sequential injections of estradiol than in controls and ewes receiving a single injection of estradiol. Treatment of ewes with a single injection of estradiol 24 or 48 h prior to necropsy failed to affect the concentration of cytoplasmic progesterone receptors. Concentrations of nuclear progesterone receptor did not differ significantly among control ewes and ewes receiving a single injection of estradiol or two sequential injections of estradiol.



A B

Figure 3

Specific binding of [^{3}H]R5020 to cytoplasmic (A) and nuclear (B) progesterone receptors in the endometria of control and estradiol-treated ewes as determined by exchange assay. Estradiol was injected 24 (1E$_2$-24), 48 (1E$_2$-48) or 24 and 48 h (2 seq. E$_2$) prior to necropsy. Endometrial samples from each of five ewes, except 1E$_2$-48 (A only) which represents four ewes, were assayed in duplicate. The concentration of cytoplasmic progesterone receptors from the omitted ewe was determined to be 1247 fmoles/mg DNA. Each bar represents the mean $\pm$ SE.

[^{3}H]Thymidine Incorporation into DNA

A single injection of estradiol 24 or 48 h prior to necropsy stimulated a small, although not statistically significant increase in incorporation of [^{3}H]thymidine into endometrial DNA (Figure 4). Significant stimulation of DNA synthesis occurred following the administration of two sequential injections of estradiol ($P<.01$).

Serum Concentrations of Progesterone

The levels of progesterone in systemic blood on Day 13 of the estrous cycle did not differ significantly among control and treated ewes (Table 2).

TABLE 2. Serum concentrations of progesterone on Day 13 of the estrous cycle

Treatment	Serum progesterone (ng/ml)
Control	2.02 ± .35[a]
1 E_2-24	2.00 ± .47
1 E_2-48	1.91 ± .60
2 seq. E_2	1.35 ± .32

[a]Mean ± SE of five ewes per group

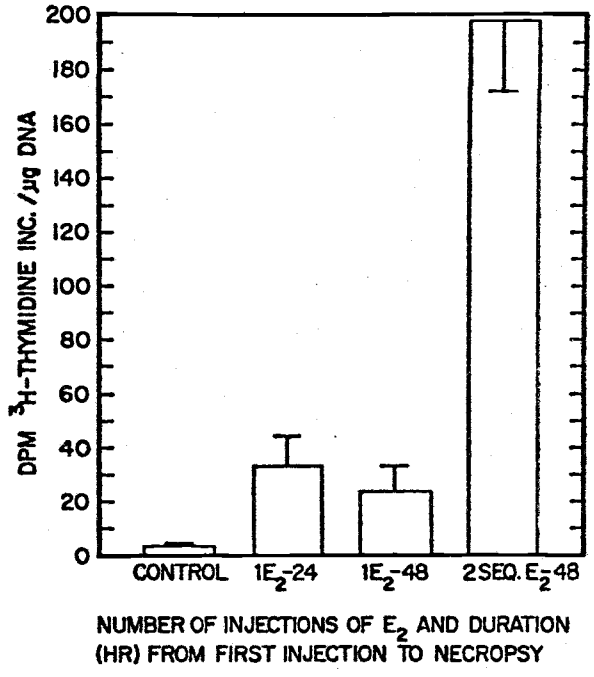


Figure 4

In vitro incorporation of labeled thymidine into endometrial DNA from control and estradiol-treated ewes. Estradiol was injected 24 ($1E_2$-24), 48 ($1E_2$-48) or 24 and 48 h (2 seq. E_2) prior to necropsy. Each bar represents the mean ± SE for five ewes.

Discussion

A synthetic progestin, R5020, was chosen for use in quantifying progesterone receptors since it binds to the receptor with high affinity (Philibert and Raynaud, 1973) and does not interact with corticosteroid binding globulin in the uterus (Philibert and Raynaud, 1973, 1974). The quantitative binding characteristics of progestin in ovine endometrium as described in this study demonstrate that concentrations of [^{3}H]R5020 equal to or greater than 1 x 10^{-8} M saturate the cytoplasmic and nuclear progesterone receptors. This observation is similar to those reported for [^{3}H]R5020 binding to progesterone receptors in the cytoplasm and nuclei of estrogen-primed rat and mouse uteri (Milgrom et al., 1977; Philibert and Raynaud, 1977; Walters and Clark, 1977). Tissue specificity of the progesterone receptor in the ewe agrees with studies conducted utilizing the rat (McGuire and Bariso, 1972; Walters and Clark, 1977), rabbit (McGuire and Bariso, 1972; Saffran et al., 1976), and guinea pig (Saffran et al., 1976). It is probable that the small amount of [^{3}H]R5020 binding observed in the spleen is not due to high affinity binding to progesterone receptors but rather to the low affinity binding of R5020 to glucocorticoid receptors in the lymphocytes (Lippman et al., 1977). Glucocorticoid receptors have not been detected in the uterus (Ballard et al., 1974).

Scatchard (1949) analyses of ligand-receptor binding in the cytoplasmic and nuclear fractions of uteri from ewes receiving two sequential estradiol injections revealed linear plots which are indicative of a single species of progesterone binding sites. The equilibrium

dissociation constants (K_d) determined for the progesterone-receptor complex in the uterine cytoplasm and nuclei of ewes receiving two sequential injections of estradiol were 1.85×10^{-9} M and 7.10×10^{-10} M, respectively. The K_d's reported in the present study are similar to those reported for uterine progesterone receptors in women (Bayard et al., 1977) and estrogen-treated rats (Walters and Clark, 1977), mice (Philibert and Raynaud, 1977), guinea pigs (Philibert and Raynaud, 1974; Feil and Bardin, 1975), and rabbits (Philibert and Raynaud, 1974). Kontula (1975) reported an association constant (K_a) of 0.91×10^{9} M for the binding of [^{3}H]-progesterone to endometrial cytoplasmic receptors of ovariectomized estradiol-treated ewes. Based on these data the K_d was calculated to be 1.10×10^{-9} M which agrees closely with the K_d for progestin binding in the cytoplasm reported herein.

In the present study a single injection of estradiol into ewes during the mid-luteal phase of the estrous cycle was without affect on concentrations of uterine cytoplasmic progesterone receptors by 48 h post-treatment while two injections of estradiol during the same interval were necessary to provoke an increase in cytoplasmic progesterone receptors and DNA synthesis. Failure of a single injection of estradiol to stimulate an increase in cytoplasmic progesterone receptors and DNA synthesis by 24 h after treatment may be due to the antagonistic effects of endogenous progesterone (Milgrom et al., 1973). Serum progesterone concentrations of ewes 24 or 48 h after a single injection of estradiol did not differ significantly from that of control ewes. Hence, it might be argued that estradiol also stimulated an increase in progesterone receptors by 48 h that was not

detected because of the ability of progesterone to autoregulate its own receptor. This seems unlikely because cytoplasmic progesterone receptors were increased by 48 h after the first of two sequential injections of estradiol yet the serum progesterone concentrations of these ewes did not differ significantly from those of animals receiving a single injection of hormone.

Absence of increased nuclear-bound progesterone in uteri of ewes 24 or 48 h after a single injection of estradiol was likely due to the ineffectiveness of treatment to stimulate an increase in cytoplasmic progesterone receptors. However, two sequential injections of estradiol provoked an increase in cytoplasmic progesterone receptors 24 h after the last injection without a concomitant increase in nuclear-bound progesterone. No explanation based upon experimental evidence can be offered at this time for the lack of increase in nuclear-bound progesterone in ewes receiving two injections of estradiol. However, since endometrial DNA synthesis was maximal at this time it might be speculated that translocation of the progesterone-receptor complex to the nucleus was somehow impaired during the S phase of the cell cycle. These results should be accepted with caution due to the low nuclear binding that was observed in endometria of control ewes and those receiving a single injection of estradiol.

Results from the present study clearly indicate the ability of exogenous estradiol to evoke increases in uterine cytoplasmic progesterone receptors in the presence of relatively high concentrations of endogenous progesterone during the mid-luteal phase of the cycle. The treatment regime and subsequent interval required for estradiol to

elicit the increases observed in this study are congruent with the post-treatment period during which the uterus acquires a luteolytic function. The ability of exogenous estradiol to stimulate the synthesis of the luteolysin by the progesterone-dominated uterus may depend upon the induction of cellular changes that are reflected by increases in cytoplasmic progesterone receptors.

EXPERIMENT II: TEMPORAL RELATIONSHIPS BETWEEN ENDOMETRIAL RNA POLYMERASE ACTIVITIES, ESTROGEN AND PROGESTERONE RECEPTORS FOLLOWING ESTRADIOL ADMINISTRATION DURING THE MID-LUTEAL PHASE OF THE OVINE ESTROUS CYCLE

Introduction

The molecular basis for the characteristic estrogen-induced uterine responses involves an interaction of the estrogen-receptor complex with the target cell genome (O'Malley and Means, 1974). Interaction of estrogen with the genome appears to be of particular importance in initiating RNA synthesis, an event that ultimately results in changes in uterine function. One of the earliest observed effects of exogenous estradiol on the rat uterus, closely correlated with the accumulation of the estradiol-receptor complex in the nucleus (Anderson et al., 1973, 1974, 1975), is an increase in RNA polymerase II activity (Glasser et al., 1972; Borthwick and Smellie, 1975; Hardin et al., 1976). Increases in high molecular weight RNA with a DNA-like composition that occur within the first hour after treatment (Knowler and Smellie, 1971; Luck and Hamilton, 1972; Borthwick and Smellie, 1975) are attributed to the increased activity of RNA polymerase II (Glasser et al., 1972; Borthwick and Smellie, 1975). An increase in RNA polymerase I activity is first detected 4 to 6 h after estradiol administration after which the activity of this enzyme remains elevated for 24 h (Borthwick and Smellie, 1975; Hardin et al., 1976). Major increases in levels of ribosomal and transfer RNA coincide with the observed increases in the activity of RNA polymerase I during these periods (Hamilton, 1968; Billing et al., 1968; Knowler and Smellie, 1971).

Studies on the early cellular events stimulated by estradiol may provide further insight into the mechanism by which this hormone stimulates the luteolytic function of the ovine uterus. Estrogen-induced luteal regression during the mid-luteal phase of the ovine estrous cycle requires the presence of the progesterone-dominated uterus (Stormshak et al., 1969; Hawk and Bolt, 1970; Warren et al., 1973; Barcikowski et al., 1974). Results of studies utilizing a treatment regime consisting of a single intramuscular injection of estradiol into ewes on each of two consecutive days suggests that the induced luteolytic function of the uterus is exerted between 24 and 48 h after the initial injection of hormone. Hysterectomy of ewes 24 h after a single injection of estradiol during mid-cycle prevents luteal regression whereas hysterectomy 48 h after the first of two sequential injections fails to block luteolysis (Chakraborty and Stormshak, 1976). Prostaglandin $F_2\alpha$ of uterine origin is recognized as the luteolysin in the ewe (McCracken et al., 1972). Two sequential injections of estradiol into ewes during mid-cycle provokes an increase in uterine synthesis and secretion of prostaglandins by 42 h after the initial injection of hormone (Ford et al., 1975). In agreement with these data, marked increases in cytoplasmic estrogen and progesterone receptors are evident in ovine endometrium 48 h after the first of two sequential injections of estradiol during this stage of the cycle, but not 24 h after a single injection of this hormone (Experiment I; Luebke et al., unpublished manuscript).

The present study was conducted to examine the temporal relationships between changes in ovine endometrial RNA polymerase activities

and estrogen and progesterone receptors during the 24 h period following the second of two sequential injections of estradiol administered during the mid-luteal phase of the estrous cycle.

Materials and Methods

Twenty-five mature crossbred ewes exhibiting normal estrous cycles of 16.3 ± 0.1 (SE) days duration, as determined by twice daily checks for estrus with vasectomized rams, were utilized for this study (first day of detected estrus = Day 0).

Ewes were assigned randomly to five groups of equal numbers. All ewes received a single intramuscular injection of estradiol-17β (500 µg dissolved in 1 ml corn oil) on each of Days 11 and 12 and were sacrificed at various time intervals following the last injection as follows: Group 1, immediately after the last injection (0 h controls); Group 2, 1 h; Group 3, 6 h; Group 4, 12 h; and Group 5, 24 h (or on Day 13).

Upon necropsy, uteri were excised, placed on ice and transported to the laboratory within 20 minutes. Uterine horns adjacent to the ovary bearing the corpus luteum were utilized. The uterine horns were split longitudinally and the endometrium was dissected from the myometrium. Further dissection was completed in a solution of 0.9% NaCl-5mM ethylene bis(oxyethylenenitrilo)tetraacetic acid (EGTA) to obtain a pool of intercaruncular endometrium from each ewe. Tissues and buffer solutions were maintained at 0-4C unless otherwise noted.

Nuclei were isolated by modifications of the procedures described by Hardin et al. (1976). Samples of intercaruncular endometrium (202.05 ± .42 mg) were homogenized in an all-glass tissue grinder

containing 2 ml of homogenization buffer (1M hexylene glycol, 100μM $MgCl_2$, 5mM EGTA, 1mM piperazine-N-N'-bis[2-ethanesulfonic acid], pH 7.5). Homogenization consisted of 30 strokes with intermittent cooling. The homogenate was centrifuged at 1500 x g for 10 min to obtain the nuclear fraction (pellet) which was resuspended in 7.5 ml homogenization buffer and recentrifuged as above. Subsequent resuspension of the pellet was carried out with 10 ml 1.9M sucrose-TKM buffer (10mM Tris-HCl, pH 7.5, 2.5mM KCl, 2mM $MgCl_2$). The suspension was transferred to a 15 ml Corex centrifuge tube, underlayed with 2 ml of sucrose-TKM and centrifuged at 15,000 x g for 50 minutes. After gentle resuspension with 2 ml TGM buffer (50mM Tris-HCl, pH 8.0, 25% glycerol, vol/vol, 1mM $MgCl_2$) using a ground glass pestle, nuclei were immediately assayed for RNA polymerase activities.

In order to determine RNA polymerase activities in nuclei isolated from ovine endometria, it was first necessary to demonstrate that the assay was linear with respect to time and concentration of DNA. Nuclei isolated from endometria of the nonpregnant uterine horn of three pregnant ewes sacrificed on Day 13 were isolated as described above. Duplicate 50 μl aliquots based on concentrations of 25, 50 or 100 mg endometrial tissue/ml buffer (approximately 10, 20 and 42 μg DNA, respectively) were added to a reaction mixture containing 12.5 μmol Tris-HCl (pH 8.0), 0.5 μmol $MgCl_2$, 0.25 μmol dithiothreitol, 0.025 μmol each of ATP, CTP and GTP (Sigma Chemical Company), 0.0025 μmol unlabeled UTP (Sigma Chemical Company) and 2.5 μCi [5-^{3}H]-uridine-5'-triphosphate (19.2 Ci/mmol, New England Nuclear) in a final volume of 250 microliters. Duplicate samples were also added to the reaction mixture plus 0.2 μg

α-amanitin (Sigma Chemical Company). Samples were incubated at 25, 30 or 37C for 15, 20 or 25 minutes. Following incubation, samples were placed on ice after the addition of 2 ml 10% trichloroacetic acid (TCA)-1% sodium pyrophosphate ($Na_4P_2O_7$). Samples were filtered through a Radnoti filter apparatus (Fisher Scientific and precipitates collected on Metricel filters (type GA-6, pore size 0.45 μm, prewashed with 25 ml 5% TCA -1% sodium pyrophosphate) which were then washed with 25 ml 5% TCA -1% sodium pyrophosphate and transferred to a soaking solution of 1M KCl - 5% TCA - 1% sodium pyrophosphate for 15 minutes. The filters were drained, dried under an infrared lamp, transferred to scintillation vials containing 10 ml scintillation fluid (0.5% 2,5-diphenyloxazole, 0.03% 2,2-p-phenylenebis [5-phenyloxazole] in toluene) and counted at 44% efficiency.

Optimal conditions, as determined, for detecting RNA polymerase activities occurred when 25 mg tissue (10 μg DNA) were incubated at 30C for 25 minutes. Activities of RNA polymerase in isolated nuclei from endometria of the 25 estradiol-treated ewes were determined as above using these conditions. It has been demonstrated that low concentrations of α-amanitin (10^{-10} to 10^{-7}M) specifically inhibit RNA polymerase II activity (Lindell et al., 1970; Novello and Stirpe, 1970). The difference between total polymerase activity (samples without α-amanitin) and polymerase I and III activities (samples containing α-amanitin) represents polymerase II activity. Although the activity resistant to α-amanitin contains both RNA polymerase I and III, it will hereafter be designated RNA polymerase I since RNA polymerase III appears to be a minor component of this activity in

nuclei isolated by the methods described above (Hardin et al., 1976).

Total specific nuclear and cytoplasmic progesterone receptors were determined by use of a [^{3}H]R5020 exchange assay as previously described (Experiment I). Samples of intercaruncular endometrium (100 mg endometrium/ml buffer) were first homogenized and centrifuged (800 x g). The resulting cytosol (supernatant) and nuclear fraction (pellet) were adjusted to 100 mg equivalents of tissue and subjected to the following procedures. Nuclear fractions were washed with buffer and incubated in duplicate with 10nM [^{3}H]R5020 (83.0 Ci/mmol, New England Nuclear) or [^{3}H]R5020 plus a 100-fold excess of unlabeled R5020 to determine total and non-specific binding, respectively. The concentration of [^{3}H]R5020 used in this study (10nM) was previously determined to saturate progesterone receptors in cytosol and nuclei of 100 mg ovine endometrium/ml buffer (Experiment I). Incubation of nuclear fractions at 4C for 20 h was followed by additional washes with buffer and extraction of bound labeled progestin with absolute ethanol. The cytosol was recentrifuged at 30,000 x g for 45 min and incubated for 20 h at 4C with the same concentrations of [^{3}H]R5020 or labeled plus unlabeled R5020 used in the exchange assay of total nuclear receptors. Following incubation the cytosol fractions were subjected to charcoal adsorption and centrifuged at 1500 x g for 45 minutes. Liquid scintillation counting of the supernatants and the ethanol extracts of the nuclear fractions was conducted to determine the concentration of total- and nonspecifically-bound progestin in the respective fractions. The difference between total and nonspecific binding represents specific binding of progestin.

Total specific nuclear estrogen receptors were estimated through an [^{3}H]estradiol exchange assay (Koligian and Stormshak, 1977b) utilizing endometrial tissue that had been quick frozen in dry ice and methanol and stored at -20C for 1 month. Briefly, 100 mg of endometrium were homogenized, centrifuged and the resulting nuclear fraction was incubated with 10nM [^{3}H]estradiol-17β (43.0 Ci/mmol, New England Nuclear) or with labeled estradiol plus a 100-fold excess of diethylstilbestrol (Sigma Chemical Company) for 30 min at 39C. Following the exchange period, the nuclear fractions were washed in buffer and the bound [^{3}H]estradiol extracted with absolute ethanol. Specific binding of labeled estradiol to the nuclear receptors was determined by difference as described above.

The DNA content of endometria from all ewes and samples of purified nuclei was quantified by the method of Burton (1956).

Data were analyzed statistically by use of one-way analyses of variance using single degree of freedom contrasts to determine the significance of differences between groups.

Results and Discussion

Two sequential injections of estradiol into ewes during the midluteal phase of the estrous cycle significantly increased ($P<.01$) the activity of RNA polymerase I in the endometrium at 6, 12 and 24 h after the second injection of hormone (Figure 1A). Although treatment with estradiol tended to increase the activity of RNA polymerase II with time, the differences among intervals were not significant statistically (Figure 1B). A transient increase in the concentration of

nuclear-bound estrogen occurred at 1 h post-treatment ($P<.01$) followed by a reduction to control levels at 12 h and a further nonsignificant increase at 24 h (Figure 2). A single injection of estradiol into the ewe during mid-cycle has been demonstrated to be ineffective in stimulating an increase in endometrial cytoplasmic estrogen receptors by 24 h (Luebke et al., unpublished manuscript). Apparently sufficient concentrations of cytoplasmic estrogen receptor were present at the time of the second injection of hormone to facilitate binding of the steroid and the subsequent translocation of the estradiol-receptor complexes to the nucleus to effect the observed increases in RNA polymerase activities. The associated patterns of changes in nuclear-bound estradiol and RNA polymerase I in the ewe are consistent with those reported to occur in the estrogen-treated immature or ovariectomized rat (Anderson et al., 1973, 1974, 1975; Borthwick and Smellie, 1975; Hardin et al., 1976).

In the estrogen-treated rat, the rapid increase in nuclear-bound estrogen in the uterus was correlated with a transient increase in the activity of RNA polymerase II after which the activity of the enzyme subsided to control levels at 2 h, increased again at 4 to 6 h and remained elevated for 24 h (Glasser et al., 1972; Borthwick and Smellie, 1975; Hardin et al., 1976). Similar marked fluctuations in the activity of RNA polymerase II in the endometrium of the estradiol-treated ewe may have occurred, but due to the sampling interval, were not detected. Alternatively, the activity of RNA polymerase II may have been stimulated by the initial injection of estradiol to such an extent that the second injection of hormone failed to evoke significant

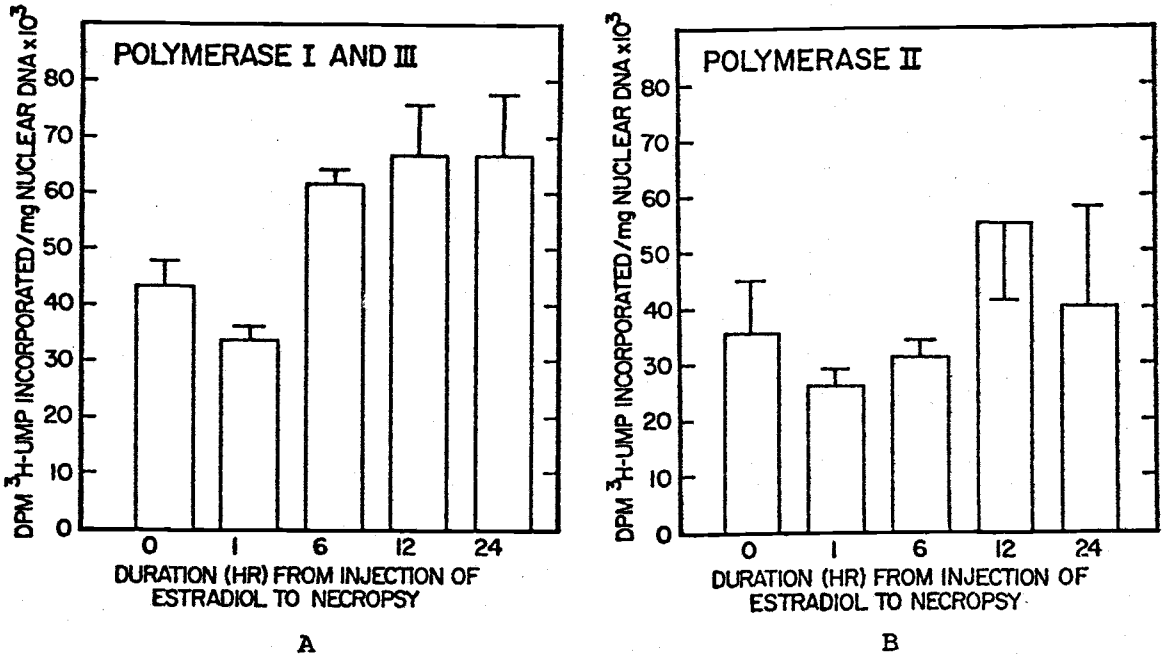


Figure 1

Activities of RNA polymerase I (A) and II (B) in endometrial nuclei of ewes receiving two sequential injections of estradiol. Ewes were sacrificed immediately (0), 1, 6, 12 and 24 h following the second injection of estradiol. Samples of isolated endometrial nuclei from each of five ewes were assayed in duplicate, with the exception of 0 and 12 h (B only) which represents three ewes and 24 h (B only) which represents four ewes. Activities were non-detectable in the omitted ewes. Each bar represents the mean $\pm$ SE.

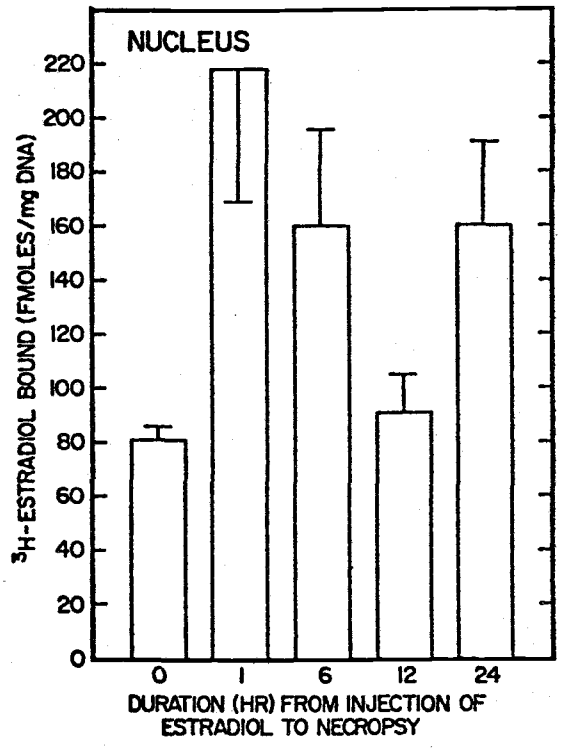


Figure 2

Specific binding of [^{3}H]estradiol to nuclear estrogen receptors in the endometria of ewes receiving two sequential injections of estradiol as determined by exchange assay. Ewes were sacrificed at various time intervals following the second injection as previously described. Endometrial samples from each of five ewes were assayed in duplicate. Each bar represents the mean $\pm$ SE.

increases in enzyme activity. Clark et al. (1978) demonstrated that increases in both RNA polymerase I and II activities were sustained for 72 h in immature rats implanted with estradiol.

Concentrations of endometrial cytoplasmic and nuclear progesterone receptors after estradiol administration to ewes are presented in Figures 3A and B, respectively. Concentrations of cytoplasmic progesterone receptors increased with time attaining a maximal level at 24 h after treatment that was greater than that of controls ($P<.05$). Differences in concentrations of cytoplasmic progesterone receptors among ewes sampled between 0 and 24 h did not differ significantly. Treatment with estradiol failed to affect concentrations of nuclear-bound progesterone in the endometria of ewes. These data are in agreement with those of Experiment I which demonstrated that an increase in concentrations of cytoplasmic progesterone receptors in the endometria of ewes occurred 24 h after the second of two sequential injections of estradiol without a concomitant increase in nuclear progesterone receptor concentrations.

Estradiol-induced synthesis of cytoplasmic estrogen and progesterone receptors in the uterus appears to be dependent upon RNA and protein synthesis. Mester and Baulieu (1975) observed that replenishment of the cytoplasmic estrogen receptor in uteri of immature rats 0 to 6 h after estradiol injection could not be blocked by cyclohexamide, an inhibitor of protein synthesis, whereas synthesis of receptors from 6 to 11 h post-treatment was dependent upon protein synthesis. Similarly, the stimulation of cytoplasmic progesterone receptor synthesis by estradiol was abolished when actinomycin D, an inhibitor

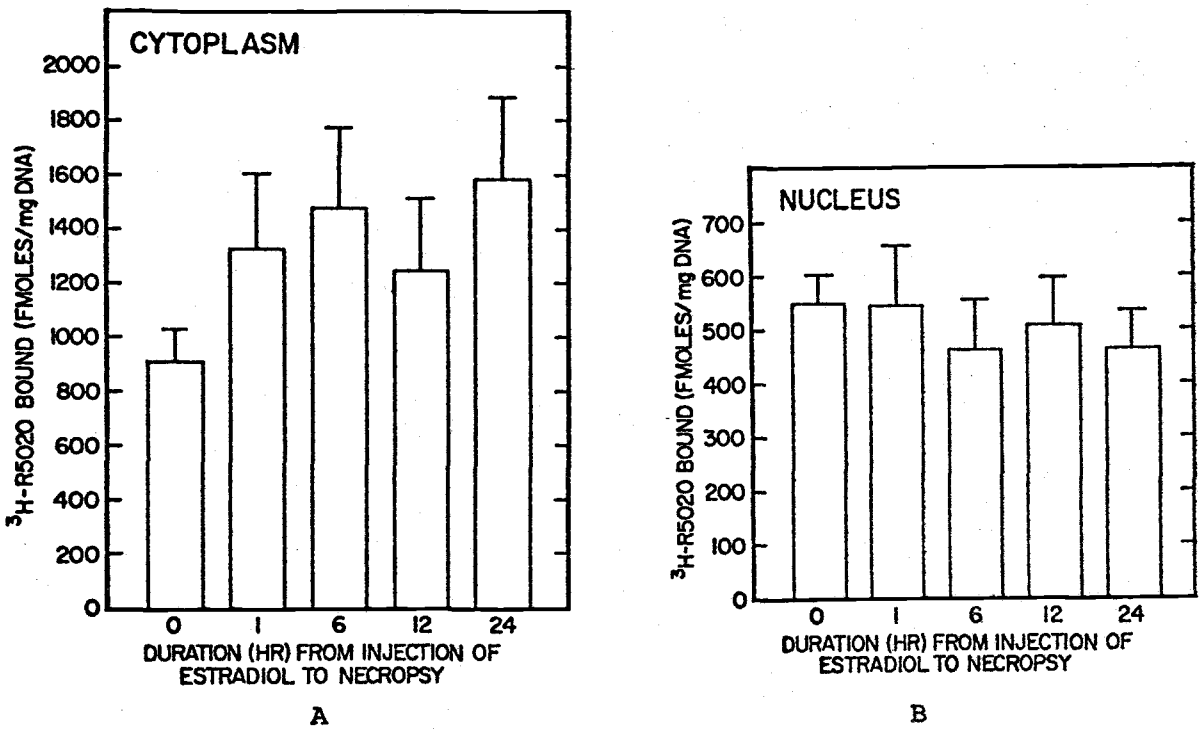


Figure 3

Specific binding of [^{3}H]R5020 to cytoplasmic (A) and nuclear (B) progesterone receptors in the endometria of ewes receiving two sequential injections of estradiol as determined by exchange assay. Ewes were sacrificed at various time intervals following the second injection of estradiol as previously indicated. Endometrial samples from each of five ewes, except 6 h (B only) which represents four ewes, were assayed in duplicate. The concentration of nuclear progesterone receptors from the omitted ewe was nondetectable. Each bar represents the mean $\pm$ SE.

of RNA synthesis, and cyclohexamide were administered 15 min before hormone injection, indicating a dependence of receptor synthesis on RNA and protein synthesis, respectively (Milgrom et al., 1973). When these inhibitors were injected 20 h or more after estradiol, cytoplasmic progesterone receptors were maintained at elevated levels (Vu Hai et al., 1977). In addition, uterine strips treated with estrogen in vitro and subsequently exposed to actinomycin D or cyclohexamide at 6 h of incubation retained elevated progesterone receptor concentrations measured at 12 h (Leavitt et al., 1977). These latter authors suggested that estrogen stimulation of RNA synthesis during the first 6 h was adequate to maintain progesterone receptor synthesis in the absence of new RNA from 6 to 12 hours. In contrast to this hypothesis, results from the present study indicate that the increase in RNA polymerase I activity beginning 6 h following the second of two sequential injections of estradiol may play a role in stimulating increased synthesis of cytoplasmic progesterone receptors. It is not known from these observations whether RNA polymerase I catalyzed the synthesis of a species of RNA that acted directly on the stimulation of receptor synthesis, or indirectly through the action of another protein.

Results from the present study indicate the ability of exogenous estradiol to stimulate an increase in RNA polymerase I activity in endometrial nuclei during a stage of the estrous cycle characterized by relatively high concentrations of endogenous progesterone. This effect of estradiol involved the interaction of the estradiol-receptor complex with the nucleus as demonstrated by the transient increase in

uterine nuclear-bound estrogen. These estradiol-induced cellular changes coincide with the post-treatment period during which the ovine uterus acquires a luteolytic function. It is conceivable that increases in RNA polymerase activities may lead to RNA synthesis that may be directly or indirectly involved with the synthesis of cytoplasmic steroid receptors and/or luteolysin by the progesterone-dominated uterus.

GENERAL DISCUSSION

Data from these experiments demonstrated the existence of a high-affinity progesterone receptor in the ovine endometrium with quantitative binding characteristics similar to those reported for progesterone receptors in uteri of laboratory animals and women. Results from these studies also indicated the ability of exogenous estradiol to evoke increases in endometrial RNA polymerse I activity and cytoplasmic progesterone receptors during a stage of the cycle characterized by relatively high concentrations of endogenous progesterone. Futhermore, this effect of estradiol involved the interaction of the estradiol-receptor complex with the nucleus as demonstrated by a transient increase in uterine nuclear-bound estrogen. The results observed in these studies coincide with the post-treatment period during which the uterus acquires a luteolytic function. The estradiol-induced increase in RNA polymerase I activity may be directly or indirectly involved with synthesis of cytoplasmic progesterone receptors and/or uterine luteolysin. Thus, these results may serve to explain, in part, the biochemical events associated with estrogen action which leads to the synthesis of the luteolysin by the progesterone-dominated uterus.

Since the ability of exogenous estradiol to induce premature luteolysis in the ewe is dependent upon prior exposure of the uterus to endogenous progesterone, further research will be required to elucidate the progesterone-induced uterine events that are requisite for the synthesis of the luteolysin. In addition, further study is

needed in order to assess the direct involvement of exogenous estradiol with synthesis of the luteolysin, possibly through the demonstration of the existence of mRNAs' or proteins specifically induced by estradiol that are required for $PGF_2\alpha$ production in the ovine uterus.

These studies may aid future researchers in their investigations of steroid hormone action in target tissues of domestic animals so that extrapolation from data obtained from the study of laboratory animals need not be employed. These studies may also provide a basis from which the actions of naturally occurring and synthetic estrogens can be investigated with regard to the regulation of target organ function and possible general somatic growth in domestic animals. Additional contributions may ultimately aid in the elucidation of substances to replace diethylstilbestrol in the rations of food-producing animals that possess growth-promoting abilities without contributing to the proliferations of tumors in human consumers.

BIBLIOGRAPHY

Aizawa, Y. and G. C. Mueller. 1961. The effect *in vivo* and *in vitro* of estrogens on lipid synthesis in the rat uterus. J. Biol. Chem. 236:281-286.

Allen, W. M. and G. W. Corner. 1930. Physiology of corpus luteum VII. Maintenance of pregnancy in rabbit after very early castration, by corpus luteum extracts. Proc. Soc. Exp. Biol. Med. 27:403-405.

Allen, E. and E. A. Doisey. 1923. An ovarian hormone: preliminary report on its localization, extraction, and partial purification, and action in test animals. J. Amer. Med. Assoc. 81:819-821.

Anderson, J. N., E. J. Peck, Jr. and J. H. Clark. 1973. Nuclear receptor-estrogen complex: relationship between concentration and early uterotropic responses. Endocrinology 92:1488-1495.

Anderson, J. N., E. J. Peck, Jr. and J. H. Clark. 1974. Nuclear receptor estradiol complex: a requirement for uterotropic responses. Endocrinology 95:174-178.

Anderson, J. H., E. J. Peck, Jr. and J. H. Clark. 1975. Estrogen-induced responses and growth: relationship to receptor estrogen binding by uterine nuclei. Endocrinology 96:160-167.

Astwood, E. B. 1938. A six hour assay for the quantitative determination of estrogen. Endocrinology 23:25-31.

Astwood, E. B. 1939a. Changes in the weight and water content of the uterus of the normal adult rat. Amer. J. Physiol. 126:162-170.

Astwood, E. B. 1939b. An assay method for progesterone based upon the decidual reaction in the rat. J. Endocrinol. 1:49-55.

Ballard, P. L., J. D. Baxter, S. J. Higgins, G. C. Rousseau and G. M. Tomkins. 1974. General presence of glucocorticoid receptors in mammalian tissues. Endocrinology 94:998-1002.

Barcikowski, B., J. C. Carlson, L. Wilson and J. A. McCracken. 1974. The effect of endogenous and exogenous estradiol-17β on the release of prostaglandin $F_2\alpha$ from the ovine uterus. Endocrinology 95:1340-1349.

Barnea, A. and J. Gorski. 1970. Estrogen-induced protein: time course of synthesis. Biochemistry 9:1899-1904.

Barrett, S., M. A. de B. Blockey, J. M. Brown, I. A. Cumming, J. R. Goding, B. J. Mole and J. M. Obst. 1971. Initiation of the oestrous cycle in the ewe by infusions of $PGF_2\alpha$ to the auto-transplanted ovary. J. Reprod. Fertil. 24:136-137.

Baulieu, E. E., M. Atger, M. Best-Belpomme, P. Corvol, J. C. Courvalin, J. Mester, E. Milgrom, P. Robel, H. Rochefort and D. DeCatalogne. 1975. Steroid hormone receptors. Vitam. Horm. 33:649-731.

Bayard, F., S. Damilano, P. Robel and E. E. Baulieu. 1978. Cytoplasmic and nuclear estradiol and progesterone receptors in human endometrium. J. Clin. Endocrinol. Metab. 46:635-648.

Bhakoo, H. S. and B. S. Katzenellenbogen. 1977. Progesterone modulation of estrogen-stimulated uterine biosynthetic events and estrogen receptor levels. Mol. Cell. Endocrinol. 8:121-134.

Billing, R. G., B. Barbiroli and R. M. S. Smellie. 1968. The early effects of oestradiol-17β on ribonucleic acid synthesis in rat uterus. Biochem. J. 109:705-706.

Blatti, S. P., C. J. Ingles, T. J. Lindell, P. W. Morris, R. F. Weaver, F. Weinberg and W. J. Rutter. 1970. Structure and regulatory properties of eukaryotic RNA polymerase. Cold Spring Harbor Symp. Quant. Biol. 35:649-657.

Bolt, D. J. and H. W. Hawk. 1972. Importance of duration of infusion of estradiol in causing CL regression in ewes. J. Anim. Sci. 35:237 (Abstract).

Borthwick, N. M. and R. M. S. Smellie. 1975. The effects of oestradiol-17β on the ribonucleic acid polymerases of immature rabbit uterus. Biochem. J. 147:91-101.

Brenner, R. M. and N. B. West. 1975. Hormonal regulation of the reproductive tract in female mammals. Ann. Rev. Physiol. 37:273-302.

Buller, R. E., D. O. Toft, W. T. Schrader and B. W. O'Malley. 1975. Progesterone-binding components of chick oviduct. VIII. Receptor activation and hormone-dependent binding to purified nuclei. J. Biol. Chem. 250:801-808.

Burton, K. 1956. A study of the conditions and mechanism of the diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. Biochem. J. 62:315-323.

Chakraborty, P. K. and F. Stormshak. 1976. Role of the uterus in oestradiol-induced luteal regression in the ewe. J. Reprod. Fertil. 48:431-432.

Chamness, G. C., A. W. Jennings and W. L. McGuire. 1973. Oestrogen receptor binding is not restricted to target nuclei. Nature 241:458-460.

Chamness, G. C., A. W. Jennings and W. L. McGuire. 1974. Estrogen receptor binding to isolated nuclei. A non-saturable process. Biochemistry 13:327-331.

Chan, L., A. R. Means and B. W. O'Malley. 1973. Rates of induction of specific translatable messenger RNAs for ovalbumin and avidin by steroid hormones. Proc. Nat. Acad. Sci. 70:1870-1874.

Chang, M. C. 1951. Maintenance of pregnancy in intact rabbits in the absence of corpora lutea. Endocrinology 418:17-24.

Clark, J. H., J. N. Anderson and E. J. Peck, Jr. 1973. Estrogen receptor-anti-estrogen complex: atypical binding by uterine nuclei and effects on uterine growth. Steroids 22:707-718.

Clark, J. H., H. A. Eriksson and J. W. Hardin. 1976. Uterine receptor-estradiol complexes and their interaction with nuclear binding sites. J. Steroid Biochem. 7:1039-1043.

Clark, J. H., J. W. Hardin, H. A. Padykula and C. A. Cardasis. 1978. Role of estrogen receptor binding and transcriptional activity in the stimulation of hyperestrogenism and nuclear bodies. Proc. Nat. Acad. Sci. 75:2781-2784.

Clark, J. H., Z. Paszko and E. J. Peck, Jr. 1977. Nuclear binding and retention of the receptor estrogen complex: relation to the agonistic and antagonistic properties of estriol. Endocrinology 100:91-96.

Clark, J. H. and E. J. Peck, Jr. 1976. Nuclear retention of receptor-estrogen complex and nuclear acceptor sites. Nature 260:635-637.

Clark, J. H. and E. J. Peck, Jr. 1977. Steroid hormone receptors: basic principles and measurement. In: W. T. Schrader and B. W. O'Malley (ed.), Laboratory Methods Manual for Hormone Action and Molecular Endocrinology. Houston Biological Associates, Houston. pp. 2-1 to 2-42.

Clark, J. H., E. J. Peck, Jr. and J. N. Anderson. 1974. Oestrogen receptors and antagonism of steroid hormone action. Nature 251:446-448.

Corner, G. W. and W. M. Allen. 1929. Physiology of the corpus luteum. II. Production of a special uterine reaction (progestational proliferation) by extracts of the corpus luteum. Amer. J. Physiol. 88:326-346.

Corvol, P., R. Falk, M. Freifeld and C. W. Bardin. 1972. In vitro studies of progesterone binding proteins in guinea pig uterus. Endocrinology 90:1464-1469.

Csapo, A. I. 1956. Progesterone "block." Amer. J. Anat. 98:273-291.

Csapo, A. I. and G. W. Wiest. 1969. An examination of the quantitative relationship between progesterone and the maintenance of pregnancy. Endocrinology 85:735-746.

DeAusa, L. J., E. Rozengurt and H. Carminatti. 1968. Estradiol induction of pyruvate kinase in the rat uterus. Biochim. Biophys. Acta 170:254-262.

DeSombre, E. R., S. Mohla and E. V. Jensen. 1972. Estrogen-independent activation of the receptor protein of calf uterine cytosol. Biochem. Biophys. Res. Comm. 48:1601-1608.

Epifanova, O. I. 1966. Mitotic cycles in estrogen-treated mice: a radioautographic study. Exp. Cell Res. 42:562-577.

Erdos, T. 1968. Properties of a uterine oestradiol receptor. Biochim. Biophys. Acta 58:932-939.

Faber, L. E., M. L. Sandmann and H. E. Stavely. 1973. Progesterone and corticosterone binding in rabbit uterine cytosols. Endocrinology 93:74-80.

Falk, R. J. and C. W. Bardin. 1970. Uptake of tritiated progesterone by the uterus of the ovariectomized guinea pig. Endocrinology 86:1059-1063.

Feil, P. D. and C. W. Bardin. 1975. Cytoplasmic and nuclear progesterone receptors in the guinea pig uterus. Endocrinology 97:1398-1407.

Feil, P. D. S. R. Glasser, D. O. Toft and B. W. O'Malley. 1972. Progesterone binding in the mouse and rat uterus. Endocrinology 91:738-746.

Ferguson, E. R. and B. S. Katzenellenbogen. 1977. A comparative study of antiestrogen action: temporal patterns of antagonism of estrogen stimulated uterine growth and effects on estrogen receptor levels. Endocrinology 100:1242-1251.

Ford, S. P., C. W. Weems, R. E. Pitts, J. E. Pexton, R. L. Butcher and E. K. Inskeep. 1975. Effects of estradiol-17β and progesterone on prostaglandins F in sheep uteri and uterine venous plasma. J. Anim. Sci. 41:1407-1413.

Freifeld, M. L., P. D. Feil and C. W. Bardin. 1974. The in vivo regulation of the progesterone receptor in guinea pig uterus: dependence on estrogen and progesterone. Steroids 23:93-104.

Giannapolous, G. and J. Gorski. 1971. Estrogen-binding protein of the rat uterus: different molecular forms associated with nuclear uptake of estradiol. J. Biol. Chem. 246:2530-2536.

Ginther, O. J. 1974. Internal regulation of physiological processes through local venoarterial pathways: a review. J. Anim. Sci. 39:550-564.

Ginther, O. J. and C. H. Del Campo. 1973. Vascular anatomy of the uterus and ovaries and the unilateral luteolytic effect of the uterus: areas of close apposition between the ovarian artery and vessels which contain uterine venous blood in sheep. Amer. J. Vet. Res. 34:1387-1393.

Ginther, O. J., C. H. Del Campo and C. A. Rawlings. 1973. Vascular anatomy of the uterus and ovaries and the unilateral luteolytic effect of the uterus: a local venoarterial pathway between uterus and ovaries in sheep. Amer. J. Vet. Res. 34:723-728.

Glasser, S. R., F. Chytil and T. C. Spelsberg. 1972. Early effects of oestradiol-17β on the chromatin and activity of the deoxyribonucleic acid-dependent ribonucleic acid polymerases (I and II) of the rat uterus. Biochem. J. 13:947-957.

Glasser, S. R. and T. C. Spelsberg. 1973. Differential modulation of estrogen induced activity of uterine nuclear RNA polymerases (I and II) by cyclohexamide and actinomycin D. Abstracts of the 55th Annual Meeting of the Endocrine Society. p. A-88.

Gorski, J. and F. Gannon. 1976. Current models of steroid hormone action: a critique. Ann. Rev. Physiol. 38:425-450.

Gorski, J. D. Toft, G. Shyamala, D. Smith and A. Notides. 1968. Hormone receptors: studies on the interaction of estrogen with the uterus. Rec. Prog. Horm. Res. 24:45-80.

Greene, G. L., L. E. Closs, H. Fleming, E. R. DeSombre and E. V. Jensen. 1977. Antibodies to estrogen receptor: immunochemical similarity of estrophilin from various mammalian species. Proc. Nat. Acad. Sci. 74:3681-3685.

Hamilton, T. H. 1968. Control by estrogen of genetic transcription and translation. Science 161:649-661.

Hamilton, T. H., C. C. Widnell and J. R. Tata. 1968. Synthesis of ribonucleic acid during early estrogen action. J. Biol. Chem. 243:408-417.

Hardin, J. W., J. H. Clark, S. R. Glasser and E. J. Peck, Jr. 1976. RNA polymerase activity and uterine growth: differential stimulation by estradiol, estriol and nafoxidine. Biochemistry 15:1370-1374.

Harris, G. S. 1971. Nature of oestrogen specific binding sites in the nuclei of mouse uteri. Nature New Biol. 231:246-248.

Harris, J. N. and J. Gorski. 1978. Estrogen stimulation of DNA-dependent DNA polymerase activity in immature rat uterus. Mol. Cell. Endocrinol. 10:293-305.

Hawk, H. W. and D. J. Bolt. 1970. Luteolytic effect of estradiol-17β when administered after midcycle in the ewe. Biol. Reprod. 2:275-278.

Henricks, D. M. and R. B. Harris, Jr. 1978. Cytoplasmic estrogen receptors and estrogen concentrations in bovine uterine endometrium. Endocrinology 103:176-185.

Hooker, C. W. and T. R. Forbes. 1947. A bioassay for minute amounts of progesterone. Endocrinology 41:158-169.

Hsueh, A. J. W., E. J. Peck, Jr. and J. H. Clark. 1976. Control of uterine estrogen receptor levels by progesterone. Endocrinology 98:438-444.

Ichikawa, S. and A. Bartoff. 1970. Tissue resistance of the progesterone-dominated rabbit uterus. Amer. J. Physiol. 219:1763-1767.

Inskeep, E. K. and R. L. Butcher. 1966. Local component of utero-ovarian relationships in the ewe. J. Anim. Sci. 25:1164-1168.

Jackson, V. and G. R. Chalkley. 1974. The binding of estradiol-17β to the bovine endometrial nuclear membrane. J. Biol. Chem. 249:1615-1627.

Janne, O., K. Kontula, R. Vihko, P. D. Feil and C. W. Bardin. 1978. Progesterone receptor and regulation of progestin action in mammalian tissues. Med. Biol. 56;225-248.

Jensen, E. V. and E. R. DeSombre. 1973. Estrogen-receptor interaction. Estrogenic hormones effect transformation of specific receptor proteins to a biologically functional form. Science 182:126-134.

Jensen, E. V. and H. I. Jacobson. 1962. Basic guides to the mechanism of estrogen action. Rec. Prog. Horm. Res. 18:387-414.

Jensen, E. V., M. Numata, P. I. Brecher and E. R. DeSombre. 1971. Hormone-receptor interaction as a guide to biochemical mechanism. Biochem. Soc. Symp. 32:133-159.

Jensen, E. V., T. Suzuki, T. Kawashima, W. E. Stumpf, P. W. Jungblut and E. R. DeSombre. 1968. A two-step mechanism for the interaction of estradiol with rat uterus. Proc. Nat. Acad. Sci. 59:632-638.

Jensen, E. V., T. Suzuki, M. Numata, S. Smith and E. R. DeSombre. 1969. Estrogen-binding substances of target tissues. Steroids 13:417-427.

Kalimi, M., S. Y. Tsai, M. J. Tsai, J. H. Clark and B. W. O'Malley. 1976. Effect of estrogen on gene expression in chick oviduct.

Correlation between nuclear-bound estrogen receptor and chromatin initiation sites for transcription. J. Biol. Chem. 251:516-523.

Kao, K. T., W. M. Arnett, and T. H. McGavack. 1969. Effect of endometrial phase, ovariectomy, estradiol, and progesterone on uterine protocollagen hydroxylase. Endocrinology 85:1057-1061.

Katzenellenbogen, B. and J. Gorski. 1972. Estrogen action in vitro. Induction of the synthesis of a specific uterine protein. J. Biol. Chem. 247:1299-1305.

Katzenellenbogen, J. A., H. J. Johnson, Jr. and K. E. Carlson. 1973. Studies on the uterine cytoplasmic estrogen binding protein: thermal stability and ligand dissociation rate. An assay of empty and filled sites by exchange. Biochemistry 12:4092-4099.

Kaye, A. M., D. Sheratzky and H. R. Lindner. 1972. Kinetics of DNA synthesis in immature rat uterus: age dependence and estradiol stimulation. Biochim. Biophys. Acta 261:475-486.

Kedinger, C., M. Gniazdowski, J. L. Mandel, Jr., F. Gissinger and P. Chambon. 1970. α-Amanitin: a specific inhibitor of one of two DNA-dependent RNA polymerase activities from calf thymus. Biochem. Biophys. Res. Comm. 38:165-171.

King, R. J. B. and J. Gordon. 1972. Involvement of DNA in the acceptor mechanism for uterine oestradiol receptor. Nature New Biol. 240:185-187.

King, R. J. B, J. Gordon, D. M. Cowan and D. R. Inman. 1966. The intranuclear localization of [6,7]-^{3}H-oestradiol-17β in dimethylbenzanthracene induced rat mammary adenocarcinoma and other tissues. J. Endocrinol. 36:139-150.

Knowler, J. T. and R. M. S. Smellie. 1971. The synthesis of ribonucleic acid in immature rat uterus responding to oestradiol-17β. Biochem. J. 125:605-614.

Kokko, E., V. Isomaa and O. Janne. 1977. Progesterone-regulated changes in transcriptional events in rabbit uterus. Biochim. Biophys. Acta 479:354-366.

Koligian, K. B. and F. Stormshak. 1977a. Progesterone inhibition of estrogen receptor replenishment in ovine endometrium. Biol. Reprod. 17:412-416.

Koligian, K. B. and F. Stormshak. 1977b. Nuclear and cytoplasmic estrogen receptors in ovine endometrium during the estrous cycle. Endocrinology 101:524-533.

Kontula, K. 1975. Progesterone-binding proteins from endometrium and myometrium of sheep uterus: a comparative study. Acta Endocrinol. 78:593-603.

Kuhn, R. W., W. T. Schrader, W. A. Coty, P. M. Conn and B. W. O'Malley. 1977. Progesterone-binding components of chick oviduct. Biochemical characterization of purified oviduct progesterone receptor B subunit. J. Biol. Chem. 252:308-317.

Lai, E. C., S. L. C. Woo, A. Dugaiczyk, J. F. Catterall and B. W. O'Malley. 1978. The ovalbumin gene: structural sequences in native chicken DNA are not contiguous. Proc. Nat. Acad. Sci. 75:2205-2209.

Leavitt, W. W. and G. C. Blaha. 1972. Estrogen-stimulated, progesterone-binding system in the hamster uterus and vagina. Steroids 19:263-274.

Leavitt, W. W., T. J. Chen and T. C. Allen. 1977. Regulation of progesterone receptor formation by estrogen. Ann. NY Acad. Sci. 282:210-223.

Leavitt, W. W., D. O. Toft, C. A. Strott and B. W. O'Malley. 1974. A specific progesterone receptor in the hamster uterus: physiologic properties and regulation during the estrous cycle. Endocrinology 94:1041-1053.

Lerner, L. J., R. Hilf, A. R. Turkheimer, I. Michel and S. L. Engel. 1966. Effects of hormone antagonists on morphological and biochemical changes induced by hormonal steroids in the immature rat uterus. Endocrinology 78:111-124.

Lindell, T. J., F. Weinberg, P. W. Morris, R. G. Roeder and W. J. Rutter. 1970. Specific inhibition of nuclear RNA polymerase II by α-amanitin. Science 170:447-449.

Lippman, M., K. Huff, G. Bolan and J. P. Neifeld. 1977. Interactions of R5020 with progesterone and glucocorticoid receptors in human breast cancer and peripheral blood lymphocytes in vitro. In: W. L. McGuire, J. P. Raynaud and E. E. Baulieu (ed.), Progesterone Receptors in Normal and Neoplastic Tissues. Raven Press, New York. pp. 193-210.

Luck, D. N. and T. H. Hamilton. 1972. Early estrogen action: stimulation of the metabolism of high molecular weight and ribosomal RNAs. Proc. Nat. Acad. Sci. 69:157-161.

Luebke, M. B., N. A. Hirota, E. J. Keenan and F. Stormshak. Influence of exogenous estradiol on endometrial progesterone and estrogen receptors during the luteal phase of the ovine estrous cycle. Submitted for publication.

Lutwak-Mann, C. 1955. Carbonic anhydrase in the female reproductive tract. Occurrence, distribution and hormonal dependence. J. Endocrinol. 13:26-38.

Lyons, W. R. 1943. Pregnancy maintenance in hypophysectomized-oophorectomized rats injected with estrone and progesterone. Proc. Soc. Exp. Biol. Med. 54:65-68.

Mapletoft, R. J. and O. J. Ginther. 1975. Adequacy of main uterine vein and ovarian artery in the local venoarterial pathway for uterine-induced luteolysis in ewes. Amer. J. Vet. Res. 36:957-963.

Mauer, H. R. and G. R. Chalkley. 1967. Some properties of a nuclear binding site of estradiol. J. Mol. Biol. 27:431-441.

McCracken, J. A., D. T. Baird and J. R. Goding. 1971. Factors affecting the secretion of steroids by the transplanted ovary in the sheep. Rec. Prog. Horm. Res. 27:537-582.

McCracken, J. A., J. C. Carlson, M. E. Glew, J. R. Goding, D. T. Baird, K. Green and B. Samuelsson. 1972. Prostaglandin $F_2\alpha$ identified as a luteolytic hormone in sheep. Nature New Biol. 238:129-134.

McGinty, D. A., L. P. Anderson and N. B. McCullough. 1939. Effect of local application of progesterone on the rabbit uterus. Endocrinology 24:829-832.

McGuire, J. L. and C. D. Bariso. 1972. Isolation and preliminary characterization of a progestogen specific binding macromolecule from the 273,000 g supernatant of rat and rabbit uteri. Endocrinology 90:496-506.

McPhail, M. K. 1934. The assay of progestin. J. Physiol. 83:145-156.

Means, A. R. and T. H. Hamilton. 1966. Early estrogen action: concomitant stimulations within two minutes of nuclear RNA synthesis and uptake of RNA precursor by the uterus. Proc. Nat. Acad. Sci. 56:1594-1598.

Means, A. R. and B. W. O'Malley. 1971. Protein biosynthesis on chick oviduct polyribosomes. II. Regulation by progesterone. Biochemistry 10:1570-1576.

Mester, J. and E. E. Baulieu. 1975. Dynamics of oestrogen-receptor distribution between the cytosol and nuclear fractions of immature rat uterus after oestradiol administration. Biochem. J. 146:617-623.

Milgrom, E. and E. E. Baulieu. 1970. Progesterone in uterus and plasma. I. Binding in rat uterus 105,000 g supernatant. Endocrinology 87:276-287.

Milgrom, E., L. Thi, M. Atger and E. E. Baulieu. 1973. Mechanisms regulating the concentration and the conformation of progesterone receptor(s) in the uterus. J. Biol. Chem. 248:6366-6374.

Milgrom, E., M. T. Vu Hai and F. Logeat. 1977. Use of [^{3}H]R5020 for the assay of cytosol and nuclear progesterone receptor in the rat uterus. In: W. L. McGuire, J. P. Raynaud and E. E. Baulieu (ed.), Progesterone Receptors in Normal and Neoplastic Tissues. Raven Press, New York, pp. 261-270.

Miller, B. G., L. Murphy and G. M. Stone. 1977. Hormone receptor levels and hormone, RNA and protein metabolism in the genital tract during the oestrous cycle of the ewe. J. Endocrinol. 73:91-98.

Mueller, G. C. 1953. Incorporation of glycine-2-^{14}C into protein by surviving uteri from α-estradiol-treated rats. J. Biol. Chem. 204:77-90.

Murdock, R. N. and I. G. White. 1966. The effect of oestradiol and progesterone on the activity of enzymes in the endometrium and caruncles of the ovariectomized ewe. J. Endocrinol. 42:187-192.

Nicolette, J. A. and J. Gorski. 1964. Effect of estradiol on glucose-U-^{14}C metabolism in the rat uterus. Arch. Biochem. Biophys. 107:279-283.

Noteboom, W. D. and J. Gorski. 1963. Early effects of estrogen and puromycin on uterine synthesis of protein, RNA and RNA polymerase. Fed. Proc. 22:329.

Notides, A. C. 1978. Conformational forms of the estrogen receptor. In: B. W. O'Malley and L. Birnbaumer (ed.), Receptors and Hormone Action, Volume II. Academic Press, New York. pp. 33-61.

Notides, A. C. and S. Nielsen. 1974. The molecular mechanism of the *in vitro* 4S to 5S transformation of the uterine estrogen receptor. J. Biol. Chem. 249:1866-1873.

Novello, F. and F. Stirpe. 1970. Simultaneous assay of RNA polymerase I and II in nuclei isolated from resting and growing rat liver with the use of α-amanitin. FEBS Lett. 8:57-60.

O'Malley, B. W., W. L. McGuire, P. O. Kohler and S. G. Korenman. 1969. Studies on the mechanism of steroid hormone regulation of synthesis of specific proteins. Rec. Prog. Horm. Res. 25:105-160.

O'Malley, B. W. and A. R. Means. 1974. Female steroid hormones and target cell nuclei. Science 183:610-620.

O'Malley, B. W., W. T. Schrader and T. C. Spelsberg. 1973. Hormone

receptor interactions with the genome of eukaryotic target cells. In: B. W. O'Malley and A. R. Means (ed.), Receptors for Reproductive Hormones. Plenum Press, New York-London. pp. 174-176.

O'Malley, B. W., R. J. Schwartz and T. C. Spelsberg. 1976. A review of regulation of gene expression by steroid hormone receptors. J. Steroid Biochem. 7:1151-1159.

O'Malley, B. W., T. C. Spelsberg, W. T. Schrader and A. W. Steggles. 1972. Mechanism of interaction of a hormone-receptor complex with a genome of a eukaryotic target cell. Nature 235:141-144.

O'Malley, B. W., D. O. Toft and M. R. Sherman. 1971. Progesterone-binding components of chick oviduct. II. Nuclear components. J. Biol. Chem. 246:1117-1122.

Pack, B. A. and S. C. Brooks. 1974. Cyclic activity of estrogen sulfotransferase in the gilt uterus. Endocrinology 95:1680-1690.

Pack, B. A., C. Christensen, M. Douraghy and S. C. Brooks. 1978. Nuclear and cytosolic estrogen receptor in gilt endometrium throughout the estrous cycle. Endocrinology 103:2129-2136.

Peck, E. J., Jr., J. Burgner and J. H. Clark. 1973. Estrophilic binding sites of the uterus. Relation to uptake and retention of estradiol in vitro. Biochemistry 12:4596-4603.

Pharriss, B. B. and L. J. Wyngarden. 1969. The effect of prostaglandin $F_2\alpha$ on the progesterone content of ovaries from psuedopregnant rats. Proc. Soc. Exp. Biol. Med. 130:92-94.

Philibert, D. and J. P. Raynaud. 1973. Progesterone binding in the immature mouse and rat uterus. Steroids 22:89-98.

Philibert, D. and J. P. Raynaud. 1974. Progesterone binding in the immature rabbit and guinea pig uterus. Endocrinology 94:627-632.

Philibert, D. and J. P. Raynaud. 1977. Cytoplasmic progestin receptors in mouse uterus. In: W. L. McGuire, J. P. Raynaud and E. E. Baulieu (ed.), Progesterone Receptors in Normal and Neoplastic Tissues. Raven Press, New York. pp. 227-243.

Pincus, G., M. C. Chang, M. X. Zarrow, E. S. E. Hafez and A. Merrill. 1956. Studies of the biological activity of certain 19-nor steroids in female animals. Endocrinology 59:695-707.

Puca, G. A., S. Vincenzo and W. Ernesto. 1974. Identification of a high-affinity nuclear acceptor site for estrogen receptor of calf uterus. Proc. Nat. Acad. Sci. 71:979-983.

Rao, B. R., W. G. Wiest and W. M. Allen. 1973. Progesterone "receptor"

in rabbit uterus. I. Characterization and estradiol-17β augmentation. Endocrinology 92:1229-1240.

Raynaud, J. P. 1977. R5020, a tag for the progestin receptor. In: W. L. McGuire, J. P. Raynaud and E. E. Baulieu (ed.), Progesterone Receptors in Normal and Neoplastic Tissues. Raven Press, New York. pp. 9-21.

Raynaud-Jammet, C., M. G. Cattelli and E. E. Baulieu. 1972. Inhibition by α-amanitin of the oestradiol-induced increase in α-amanitin sensitive RNA polymerase in immature rat uterus. FEBS Lett. 22: 234-237.

Roeder, R. G. and W. J. Rutter. 1969. Multiple forms of DNA-dependent RNA polymerase in eukaryotic organisms. Nature 224:234-237.

Roeder, R. G. and W. J. Rutter. 1970. Specific nucleolar and nucleoplasmic RNA polymerases. Proc. Nat. Acad. Sci. 65:675-682.

Saffran, J., B. K. Loeser, S. A. Bohnett and L. E. Faber. 1976. Binding of progesterone receptor by nuclear preparations of rabbit and guinea pig uterus. J. Biol. Chem. 251:5607-5613.

Sarff, M. and J. Gorski. 1971. Control of estrogen binding protein concentration under basal conditions and after estrogen administration. Biochemistry 10:2557-2563.

Scatchard, G. 1949. The attractions of proteins for small molecules and ions. Ann. NY Acad. Sci. 51:660-672.

Schrader, W. T., S. S. Heuer and B. W. O'Malley. 1975. Progesterone receptors of chick oviduct: identification of 6S receptor dimers. Biol. Reprod. 12:134-142.

Schrader, W. T., R. W. Kuhn and B. W. O'Malley. 1977. Progesterone-binding components of chick oviduct. Receptor B subunit protein purified to apparent homogeneity from laying hen oviducts. J. Biol. Chem. 252:299-307.

Schrader, W. T. and B. W. O'Malley. 1972. Progesterone binding components of chick oviduct. IV. Characterization of purified subunits. J. Biol. Chem. 247:51-59.

Schrader, W. T. and B. W. O'Malley. 1978. Molecular structure and analysis of progesterone receptors. In: B. W. O'Malley and L. Birnbaumer (ed.), Receptors and Hormone Action, Volume II. Academic Press, New York. pp. 189-224.

Schwartz, R. J., C. Chang, W. T. Schrader and B. W. O'Malley. 1977. Effect of progesterone receptors on transcription. Ann. NY Acad. Sci. 286:147-160.

Senior, B. E. 1975. Cytoplasmic oestradiol-binding sites and their relationship to oestradiol content in the endometrium of cattle. J. Reprod. Fertil. 44:501-511.

Senior, M. B. and F. R. Frankel. 1978. Evidence for two kinds of chromatin binding sites for the estradiol-receptor complex. Cell 14:857-863.

Sherman, M. R., P. L. Corvol and B. W. O'Malley. 1970. Progesterone-binding components of chick oviduct. I. Preliminary characterization of cytoplasmic components. J. Biol. Chem. 245:6085-6096.

Shyamala, G. and J. Gorski. 1969. Estrogen receptors in the rat uterus: studies on the interaction of cytosol and nuclear binding sites. J. Biol. Chem. 244:1097-1103.

Smith, E. R. and K. L. Barker. 1974. Effects of estradiol and nicotinamide adenine dinucleotide phosphate on the rate of synthesis of uterine glucose-6-phosphate dehydrogenase. J. Biol. Chem. 249:6541-6547.

Spaziani, E. and C. M. Szego. 1958. The influence of estradiol and cortisol on uterine histamine of the ovariectomized rat. Endocrinology 63:669-678.

Spelsberg, T. C. and R. F. Cox. 1976. Effects of estrogen and progesterone on transcription, chromatin and ovalbumin gene expression in the chick oviduct. Biochim. Biophys. Acta 435:376-390.

Spelsberg, T. C., A. W. Steggles, F. Chytil and B. W. O'Malley. 1972. Progesterone-binding components of chick oviduct. V. Exchange of progesterone-binding capacity from target to nontarget tissue chromatin. J. Biol. Chem. 247:1368-1374.

Spelsberg, T. C., A. W. Steggles and B. W. O'Malley. 1971. Progesterone-binding components of chick oviduct. III. Chromatin acceptor sites. J. Biol. Chem. 246:4188-4197.

Stancel, G. M., K. M. T. Leung and J. Gorski. 1973. Estrogen receptors in the rat uterus. Relationship between cytoplasmic and nuclear forms of the estrogen binding protein. Biochemistry 12:2137-2141.

Stone, G. M., L. Murphy and B. G. Miller. 1978. Hormone receptor levels and metabolic activity in the uterus of the ewe: regulation by oestradiol and progesterone. Austr. J. Biol. Sci. 31:395-403.

Stormshak, F., H. E. Kelley and H. W. Hawk. 1969. Suppression of ovine luteal function by 17β-estradiol. J. Anim. Sci. 29:476-478.

Stormshak, F., R. Leake, N. Wertz and J. Gorski. 1976. Stimulatory and inhibitory effects of estrogen on uterine DNA synthesis. Endocrinology 99:1501-1511.

Szego, C. M. and S. Roberts. 1953. Steroid action and interaction in uterine metabolism. Rec. Prog. Horm. Res. 8:419-468.

Teng, C. and T. H. Hamilton. 1969. Role of chromatin in estrogen action in the uterus. II. Hormone-induced synthesis of non-histone acidic proteins which restore histone-inhibited DNA-dependent RNA synthesis. Proc. Nat. Acad. Sci. 63:465-472.

Toft, D. O. 1972. The interaction of uterine estrogen receptors with DNA. J. Steroid Biochem. 3:515-522.

Toft, D. and J. Gorski. 1966. A receptor molecule for estrogens: isolation from the rat uterus and preliminary characterizations. Proc. Nat. Acad. Sci. 55:1574-1581.

Tomkins, G. M. 1970. Regulation of specific protein synthesis in eukaryotic cells. Cold Spring Harbor Symp. Quant. Biol. 35:636-640.

Trams, G., H. Brewitt, H. Mollman and H. Maas. 1973. Effect of progesterone on RNA and protein synthesis in the rat uterus. Acta Endocrinol. 73:740-750.

Tsai, S., M. J. Tsai, R. Schwartz, M. Kalimi and J. H. Clark. 1975. Effects of estrogen on gene expression in chick oviduct: nuclear receptor levels and initiation of transcription. Proc. Nat. Acad. Sci. 72:4228-4232.

Tseng, L. and E. Gurpide. 1975. Induction of human endometrial estradiol dehydrogenase by progestins. Endocrinology 97:825-833.

Vu Hai, M. T., F. Logeat, M. Warembourg and E. Milgrom. 1977. Hormonal control of progesterone receptors. Ann. NY Acad. Sci. 286:199-207.

Walters, M. R. and J. H. Clark. 1977. Cytosol progesterone receptors of the rat uterus: assay and receptor characteristics. J. Steroid Biochem. 8:1137-1144.

Warren, J. E., H. W. Hawk and D. J. Bolt. 1973. Evidence for progestational priming of estradiol-induced luteal regression in the ewe. Biol. Reprod. 8:435-440.

Weinman, R. and R. G. Roeder. 1974. Role of DNA-dependent RNA polymerase III in transcription of the tRNA and 5S RNA genes. Proc. Nat. Acad. Sci. 71:1790-1794.

West, N. B., H. G. Verhage and R. M. Brenner. 1976. Suppression of the estradiol receptor system by progesterone in the oviduct and uterus of the cat. Endocrinology 99:1010-1016.

Williams, D. and J. Gorski. 1974. Equilibrium binding of estradiol by uterine cell suspensions and whole uteri *in vitro*. Biochemistry 13:5537-5542.

Wiltbank, J. N. and L. E. Casida. 1956. Alteration of ovarian activity by hysterectomy. J. Anim. Sci. 15:134-140.

Yamomoto, K. R. and B. M. Alberts. 1975. The interaction of estradiol-receptor protein with the genome: an argument for the existence of undetected specific sites. Cell 4:301-310.

Yamomoto, K. R., U. Gehring, M. R. Stampfer and C. H. Sibley. 1976. Genetic approaches to steroid hormone action. Rec. Prog. Horm. Res. 32:3-32.