

Estrogen Action and Genetic Expression in the Uterus

Gerald C. Mueller

McArdle Laboratory  
University of Wisconsin  
Madison, Wisconsin 53706

In the process of differentiation the conditions for expressing certain genetic potentials are determined for each cell giving rise to the spectrum of cell types which comprise the mammalian organism. When the product of one cell type selectively modifies the function of another we refer to the communicating molecule as a hormone and the responding unit as the target cell. Thus the process of differentiation has vested in the ovary the cyclic production of estrogens which in turn controls function in distant sites as the uterus, vagina, hypothalamic-pituitary axis, and the mammary gland. This presentation is concerned with the molecular mechanisms which operate in the response of cells to estrogenic hormones. The thesis will be developed that a molecule of estrogen, through interaction with a specific receptor, a macromolecular aggregate in the target cell, activates a process which is otherwise rate limiting to the genetic expression mechanism of that cell. In this manner the synthesis of new ribonucleic acids and proteins is induced; these lead to the acceleration of anabolic processes and the cumulative modification of cell functions which constitute the hormone response. At the molecular level estrogen action is viewed as a study of the biology of interacting macromolecular aggregates through which the availability of factors necessary for the activity of DNA-dependent RNA polymerase of the nucleus is regulated.

#### Early Uterine Changes in Response to Estradiol

While estrogenic hormones effect a variety of responses in a spectrum of tissues the best understood is perhaps the response of the rat uterus. In the absence of estrogens this organ atrophies and exhibits minimal metabolic activity with respect to the synthesis of RNA, protein, and phospholipids; cell replication is also remarkably restricted. The administration of exogenous estradiol, however, produces a rapid reversal of this state and the anabolic shifts are soon reflected in the composition of the uterus. The response is

initially one of hypertrophy with a striking accumulation of phospholipids and imbibition of water in the first few hours of estrogen action; the levels of RNA and protein rise somewhat later (Aizawa and Mueller, 1961) (Fig. 1). The replication of cells as measured by changes in DNA content of the uterus is delayed for approximately 72 hours.

Even though the changes in gross composition suggest a sequential effect of the hormone on the respective metabolic pathways this may be more apparent than real. Incorporation studies using radioactive precursors either in the animal or in surviving uterine segments in vitro illustrate that the synthesis of RNA, protein and phospholipids are all stimulated nearly simultaneously (Ui and Mueller, 1963; Noteboom and Gorski, 1963; Gorski and Nicolette, 1963; Aizawa and Mueller, 1961).

Examples of the early accelerating action of estradiol on the synthesis of phospholipids and proteins are shown in Figures 2 and 3. Similarly, Hamilton (1965) demonstrated an accelerating effect of estradiol treatment on RNA synthesis as early as 30 minutes.

In an attempt to find an explanation for the acceleration of metabolic activity a number of representative enzymes were assayed. The activities of amino acid activating enzymes (McCorquodale and Mueller, 1958), serine aldolase (Herranen and Mueller, 1957) and RNA polymerase (Gorski, 1964) were all increased within the first few hours of estrogen treatment. These results suggested that the increased activity of enzymes was indeed responsible for the acceleration of the synthetic pathways.

While these results implied that the hormone responses depended on the synthesis of new metabolic machinery it remained for experiments with the antibiotics, puromycin and actinomycin D, to show this dependence and to point up the role of estrogens in modifying genetic expression mechanisms in

the uterine cell. Levels of puromycin which inhibited 90 percent of the protein synthesis in the uterus in vivo, blocked the estrogen-induced alterations of RNA and phospholipid synthesis as well as the early imbibition of water (Fig. 4) (Mueller, Gorski and Aizawa, 1961). Gorski and Axman (1964) extended this type of study using cycloheximide, another inhibitor of protein synthesis, and confirmed that the early hormonal effects depend on protein synthesis.

Similarly levels of actinomycin D that reduce the in vivo synthesis of RNA in the uterus to below 10 percent of the control value prevented the acceleration of protein and phospholipid synthesis by estradiol (Fig. 5). The imbibition of water, however, was less affected by the antibiotic and about 30 percent of the wet weight response remained even with massive doses of actinomycin D which abolished RNA synthesis (Ui and Mueller, 1963).

That a fraction of the estrogen-induced protein synthesis was insensitive to the action of actinomycin D was further shown using adrenalectomized animals (Nicolette and Mueller, 1966b). In accord with the basic observations of Lippe and Szego (1965) it was observed that adrenalectomy makes more prominent that portion of the early estrogen response which is independent of the synthesis of new RNA (Fig. 6). In the adrenalectomized animals this was shown to include a significant stimulation of protein synthesis. The existence of a fraction of the anabolic response which is independent of RNA synthesis clearly points to a primary action of the hormone at a site distant from the genome. However, it is clearly shown in these studies that in addition to any action of the hormone at an extragenomic site, the amplification of the primary action depends strikingly on the usual mechanisms of genetic expression, the synthesis of new RNA and protein.

The possibility of a separate and additional action of estrogens at a specific gene site, such as has been suggested for ecdysone (Karlson, 1963), remains a possibility, which has not been tested in these experiments.

The observation that uterine protein synthesis could be significantly accelerated by estrogens, even though RNA synthesis was blocked by actinomycin D, demonstrates that the hormone can facilitate the use of pre-existing messenger RNA molecules as well as the cell's pre-existing machinery for synthesizing proteins. Insight into the molecular mechanisms by which this is accomplished now appears fundamental to our understanding of the initial action of the hormone in the uterine cell. In fact, it is logical to divide the hormone response with this phenomenon into the primary or signal event which uses pre-existing cell machinery and the amplification process which increases the transcription of genetic information into newly synthesized RNA and the subsequent translation of this information into the construction of the protein machines of the cell.

Some Characteristics of the Interaction of Estradiol with the Receptor in Target Cells

The ability of estrogens to elicit dramatic and selective responses in organs as the uterus, vagina and mammary gland has long suggested that the cells of these tissues might contain a specific receptor for the hormone. The pioneer efforts of Jensen and his associates (Jensen and Jacobson, 1960; Jensen, 1965; Jensen, DeSombre and Jungblut, 1966) have provided a solid molecular basis for this concept. Using tritiated estradiol of very high specific activity he demonstrated that the uterus and vagina selectively concentrate the hormone against a concentration gradient (Fig. 7). Fractionation attempts in a number of laboratories (Talwar, Segal, Evans and Davidson, 1964; Noteboom and Gorski, 1965; Toft and Gorski, 1966) have shown that the receptor

is protein in part and is distributed between the nuclear and cytoplasmic fractions of uterine homogenates. Centrifugal analysis revealed the soluble receptor to have a sedimentation constant of 9.55 (an approximate molecular weight of 200,000); the receptor released from the nuclear fraction with 0.3 M KCl had an S value of 4.55.

While the localization to target organs and the high selectivity of the receptor for active estrogens points to the possibility that the receptor signals the hormone response, the actual proof of this role rests on the elucidation of the nature of the signal and showing its relation to the acceleration of the genetic expression mechanisms. To this point our laboratory has studied the binding of tritiated estradiol to the soluble receptor of uterine homogenates. In practice homogenates of rat uteri prepared in a solution of 0.01 M TRIS, 0.01 M KCl and 0.0015 M MgCl<sub>2</sub> are centrifuged at 100,000 X G for 60 minutes and the soluble phase passed over a P-200 Biogel column. In agreement with gel filtration studies of Talwar, Segal, Evans and Davidson (1964) the estrogen receptor, with or without bound hormone, was eluted in the first peak. To study the binding process in vitro the tritiated hormone was added to the solution of the receptor and the amount of bound hormone determined after passing the reaction mixture over a column of P-10 Biogel to remove the free hormone (Vonderhaar and Mueller, 1967).

The fact that the soluble receptor exists in more than one physical state was revealed in a study of the binding at different temperatures. At 0° only one-third to one-half as much binding occurred as at 23° (Fig. 8). It was also shown that after the binding at 0° was nearly complete, raising the temperature to 23° resulted in an additional fraction of hormone being bound. It was concluded from these data that, not only does the receptor exist in more than one form, but that a temperature sensitive process is necessary for a certain fraction of the receptors to bind hormone. Experiments to separate

a temperature sensitive process from the actual binding process have shown that the simultaneous presence of estradiol and the elevated temperature are necessary. This is of interest as it suggests that the hormone cooperates in the temperature dependent process leading to the extra binding.

Looking for factors which might modify the binding reaction a group of nucleotides and cofactors have been examined. Among these substances 3'5' cyclic AMP was found to accelerate the binding at 23°*C*. However, comparison of other adenine nucleotides revealed that this effect was not confined to the cyclic AMP (Fig. 9) since 5'AMP and 5'ATP worked as well over concentration ranges from 10<sup>-6</sup>*M* to 10<sup>-3</sup>*M*. In preliminary studies with other nucleotides and bases it appears that the primary effect is mediated by the adenine base and that other nucleotides such as UTP, CTP and GTP have little effect.

These experiments also show that the estrogen-receptor complex is not a stable one since in most instances continued incubation led to the loss of the bound hormone in the assay procedure used. The addition of ATP after most of the binding had taken place appeared to delay or prevent this loss (Fig. 9). However, once the loss of the bound form was initiated it continued at nearly the same rate whether the reaction mixture was kept at 23° or 0°. Thus the binding process at 23° is a summation of two reactions: - one leading to the binding and another changing the bound form so that it no longer is recovered in the eluate of the P-10 column used in the assay. Preliminary studies suggest that the receptor complexes begin to aggregate after binding hormone and may adsorb on the column. In any case these experiments suggest that the receptor undergoes some transformation while in contact with the hormone and that this transformation may be intrinsic to the molecular action of the hormone.

Some insight into this transformation and the character of the estrogen receptor might be obtained if one could isolate the receptor free of the

other constituents in the P-200 front. Towards this end two types of resins have been synthesized bearing estradiol molecules immobilized on their surfaces: one of these has a back-bone of aminobenzyl cellulose to which the estrogen is coupled as an azo compound (PAB-estradiol) (Fig. 10) and the other is a polystyrene resin with maleimide groups to which estradiol was attached through  $17\alpha$  propyl mercaptan side chain (PVM-estradiol) (Fig. 11). Corresponding control resins were also prepared with the appropriate sub-groups as indicated. The PAB-estradiol was prepared and studied independently in Dr. Jensen's laboratory (Jensen, DeSombre and Jungblut, 1966). While the binding appears to be somewhat different with the two resins, both materials are highly selective in the removal of the receptor from solution. Using these resins at  $0^{\circ}$  it appears that only a fraction of the receptors is removed, the extra receptors pass through the column and can be assayed for in the filtrate. In these experiments it was noted with both PAB-estradiol and PVM-estradiol that more receptor was removed when a small amount of RNase was present in the medium. This partial dependency of the binding on action of RNase is shown more clearly by adsorbing first with PVM-estradiol and then adding graded levels of RNase in the binding of free estradiol to the receptor remaining in the filtrate (Fig. 12). Carboxymethylated RNase did not substitute for the active enzyme. These findings suggest that the extra binding was achieved through a digestion of some RNA in the receptor preparation which was restrictive to the binding process.

Taken together these studies indicate that the estrogen receptor from the soluble fraction of the cell exists in more than one physical form and that certain potential receptors are unable to bind the hormone unless the temperature is raised to  $23^{\circ}$ . The change which ensues is somehow sensitive to the presence of adenine nucleotides and sets in operation a process which

ultimately changes the character of the receptor. That ribonucleic acid may be a part of the restricted receptor is inferred by the finding that low levels of RNAse facilitate the binding of estradiol. It is proposed that the hormone receptor is really an aggregate which in some instances contains RNA in a combination which is restrictive to the binding process. Recent experiments have shown that the solution containing the receptor is able to stimulate a protein synthesizing system from HeLa cells which has been staged so as to be deficient in messenger RNA; this activity was decreased by prior-contact of the receptor solution with the estradiol resins. While this presents an interesting role for the RNA these results are completely preliminary and must be regarded as only suggestive.

#### RNA Polymerase Activity in the Control of Genetic Expression by Estrogens

The study of the sequence of molecular events set in motion by estradiol has demonstrated that the acceleration of RNA synthesis is a basic requirement in the amplification of the early estrogen action. Data from our laboratory (Ui and Mueller, 1963; Mueller, 1965) and from Gorski and Nelson (1965) show that the induced RNA synthesis involves all major classes of RNA although the majority of the RNA synthesized appears to be ribosomal. Greenman and Kenney (1964), in fact, have shown that uterine ribosomes increased greatly in amount during the first four hours of estrogen action and that these ribosomes were also charged to a greater extent with messenger RNA than were ribosomes of control uteri. Moore and Hamilton (1964) have confirmed and extended these observations. Accordingly, any proposed mechanism of estrogen action has to explain the accelerating effect of the hormone on RNA synthesis, an accelerating effect which is basic to the synthesis of all major classes of RNA in the rat uterus.

One possibility for such regulation arises from the close coupling of RNA and protein synthesis in mammalian cells. Using cultures of HeLa cells it was shown previously (Tamaoki and Mueller, 1965a, 1965b) that newly synthesized ribosomal precursor RNA is combined with newly synthesized protein as a ribonucleoprotein intermediate in the assembly of ribosomes. Since levels of puromycin which inhibit protein synthesis also greatly depress RNA synthesis it would appear that the combination with protein to form a ribonucleoprotein is an obligate feature of RNA synthesis in the mammalian nucleus. In a mechanistic sense the formation of a ribonucleoprotein might provide the means for removing the newly synthesized RNA from the polymerase site and cyclic reactivation of polymerase. It is conceivable that the hormonal regulation of RNA synthesis could be exerted at this level.

To explore this possibility we undertook a study of the role of protein synthesis in the regulation of RNA polymerase activity in nuclei. As previously cited the acceleration of uterine RNA synthesis by estrogen is closely correlated with an increased activity of the DNA-dependent RNA polymerase of the isolated nuclei (Fig. 13). When the uteri are removed from the animals and incubated in vitro in Eagles' tissue culture medium the estrogen induced polymerase activity is maintained. However, adding cycloheximide to the tissue culture medium in which the surviving uteri are incubated rapidly depresses the RNA polymerase activity to the level of the control nuclei (Fig. 14). In contrast the initial low polymerase activity of control nuclei is not affected by the cycloheximide treatment (Nicolette and Mueller, 1966a; Nicolette, LeMahieu and Mueller). This finding is taken as evidence that estrogen treatment in/living animal activates a fraction of RNA polymerase which requires continued protein synthesis for its activity. The sensitivity of the estrogen-induced RNA polymerase to blockade of protein synthesis in

these studies is confirmed by similar studies of Gorski in the whole rat (Gorski, Noteboom and Nicolette, 1965). Whether the primary action of the hormone is to activate a process supplying an essential protein to the polymerase site or whether it activates the RNA polymerase directly which then requires protein for successful function is difficult to discern from these experiments; however, the former possibility is favored.

During the course of these studies yet another interesting property of the estrogen induced polymerase was revealed. It was observed that the polymerase level of uteri from estrogen-treated animals was retained on incubation of the surviving uteri in the tissue culture medium as long as the incubation temperature was maintained at 37°*C*. However, if the incubation temperature was reduced to 23°*C* the polymerase level in the nuclei of the surviving uteri was depressed rapidly to the control level. Again the polymerase of nuclei from control uteri was remarkably insensitive to this mild treatment (Fig. 15). The process leading to the reduction in polymerase activity in estrogenized uteri appears to be an active one and also dependent on a temperature sensitive step since dropping the incubation temperature to 4°*C* kept the estrogen induced polymerase at the initial level. It should be emphasized that in all of these experiments the assay of polymerase activity was carried out at 37°*C* in nuclei isolated from the pre-incubated uteri.

Return of the incubation temperature to 37°*C* after incubation of the uteri for 1-2 hours or longer at 23°*C* was associated with a rapid regain of polymerase activity (Fig. 15 and 16). However the return of polymerase activity in estrogenized uteri was only partially sensitive to the blockade of protein synthesis with cycloheximide. In the case of control tissues raising the temperature of the incubation to 37°*C* was also associated with a small but significant surge of polymerase activity. This was entirely prevented

with cycloheximide (Nicolette and Mueller, 1966a; Nicolette, LeMahieu and Mueller).

Taken together these data support the conclusion that a large fraction of the RNA polymerase of the rat uterus requires the availability of some recently synthesized protein for continued function and that in its absence the polymerase becomes inactive. It also appears that this essential protein is used up or inactivated during the course of polymerase action since the polymerase is reversibly inactivated. This relationship is compatible with the original proposal (Mueller, 1966) that the protein might be used to capture newly synthesized RNA as a ribonucleoprotein.

If the decline of polymerase activity in nuclei of uteri incubated with cycloheximide was in fact due to the exhaustion of a protein which is used up in polymerase action it should be possible to protect against the drop in polymerase activity by preventing RNA synthesis during incubation of the uteri with cycloheximide. Until recently an experiment to test this point was not possible but the discovery of an inhibitor of RNA synthesis (Buchnall and Carter, 1967) (MPB) (Fig. 17) which is reversible (Summers and Mueller) opens the way for this and other exciting experiments on the regulation of polymerase activity. This agent while highly effective in blocking RNA synthesis in the intact tissue is easily reversed on transfer of the tissue to an MPB-free medium. Furthermore, nuclei from inhibited tissues exhibit full RNA polymerase activity immediately on isolation from the treated cells. Using this agent to block RNA synthesis in the surviving uteri incubated in vitro in tissue culture medium it was shown that the depression of RNA polymerase activity by cycloheximide was completely prevented; in fact the RNA polymerase activity of nuclei isolated from uteri incubated with both cycloheximide and MPB was higher than the starting level in the estrogenized tissue (Fig. 18).

Subjecting both control and estrogen treated uteri to incubation with MPB and cycloheximide raised the polymerase level of nuclei from both tissues to the same high level (Fig. 19). Since these levels were attained in the presence of sufficient cycloheximide to block protein synthesis, one must conclude that the increased polymerase activity constitutes an activation of pre-existing polymerase in both cases and that the absolute amount of potential polymerase activity was the same in both control and estrogen treated tissues. This argues that the early increase of polymerase activity in the living animal following estradiol treatment is primarily an activation of pre-existing polymerase (Mueller and LeMahieu).

Since polymerase activity has been shown in these studies to be very closely tied to the availability of a protein which appears to be used up in the course of polymerase action, it appears that in these experiments with both cycloheximide and MPB a sufficient supply of the protein must exist in the cells of both control and estrogen treated uteri to activate the polymerase if RNA synthesis is prevented. While this amount might be very small indeed, its form and function is of very great interest. Attempts to assay the rate of production and total availability of the protein using these two agents are under way.

#### A Hypothesis of Estrogen Action

While our understanding of estrogen action is still limited and obscured by the vast complexity of cells, a general picture begins to emerge which relates some of the interacting cellular components (Fig. 20). In this picture the initial step in hormone action is visualized as the combination of the estrogen molecules with a specific receptor protein. It is postulated that this protein is itself a product of specific gene action and that a separate

set of factors regulate the expression of this gene in selectively differentiated cells. In the target cells of the rat uterus, this protein already exists; however, it appears to reside in molecular aggregation with other cellular components. The binding studies suggest that some receptors are complexed with RNA since RNA plays a role in the hormone binding process. Preliminary isolation studies also support this view since partially purified preparations of hormone receptor stimulated amino acid incorporation of a messenger deficient system and this activity can be reduced by prior adsorption with resins containing immobilized estradiol.

Another fraction of the receptors are unable to bind hormone unless assisted by some temperature sensitive mechanism in which adenine compounds also play a role. Furthermore, once the receptor has combined with estradiol it appears to undergo a transformation leading to the loss of the bound hormone in the assay procedure which was used. Taken together these observations prompt the hypothesis that the combination of the hormone with the receptor complex induces an allosteric modification of the complex so as to expose or activate certain components. This could be a cyclic process in which case the receptor could be visualized as an instrument for selective transport of a macromolecular entity.

The possibility that messenger RNA may be released by such action for use by the synthetic machinery of the cell is suggested by the observation that protein synthesis is accelerated by in vivo hormone treatment even though RNA synthesis had been blocked with actinomycin D. In accord with this concept Notides and Gorski (1966) have shown that the synthesis of certain uterine proteins is stimulated selectively under such circumstances.

While this picture provides a molecular mechanism for the primary hormonal signal it is equally important to relate the signal to the amplification process which involves an acceleration of genetic expression. In this case the high

dependency of the estrogen-induced RNA polymerase on the synthesis or availability of protein suggests a possible basis for coupling these aspects of hormone action. As a working hypothesis it is proposed that the action of the hormone on the receptor causes more of an essential protein to be synthesized and made available to the polymerase site where it supports the cyclic action of this enzyme. With the accelerated production of RNA both the information and the building blocks are elaborated which provide for the anabolic changes which we recognize as the hormone response. The specific character of the response, however, can be expected to vary according to the manner in which a given cell has been programmed during its differential development. It should also be kept in mind that the initial action of the hormone may lead to the early synthesis of more than one type of protein and that certain of these new proteins may interact with metabolic systems other than the polymerase system.

#### Acknowledgement

This paper presents a summation of the efforts of a number of past and present scientific associates. I am indeed grateful for their contributions which have been recorded in detail elsewhere. With respect to current investigators I would like to credit Miss Barbara Vonderhaar for the estrogen binding studies, Miss Mary LeMahieu and Dr. John Nicolette for the studies on induced alterations of RNA polymerase level, and Dr. Wilma Summers for the comparative studies of MPB action in HeLa nuclei. This work was supported by grants CA-07175, 5-K6-CA-685 from the National Cancer Institute.

## REFERENCES

- Aizawa, Y. and Mueller, G. C. (1961): The effect in vivo and in vitro of estrogens on lipid synthesis in the rat uterus. *Journal of Biological Chemistry*, 236, 381.
- Bucknall, R. A. and Carter, S. B. (1967): A reversible inhibitor of nucleic acid synthesis. *Nature*, 213, 1099.
- Gorski, J. (1964): Early estrogen effects on the activity of uterine ribonucleic acid polymerase. *Journal of Biological Chemistry*, 239, 889.
- Gorski, J. and Axman, M. C. (1964): Cycloheximide (actidione) inhibition of protein synthesis and the uterine response to estrogen. *Archives of Biochemistry and Biophysics*, 105, 517.
- Gorski, J. and Nelson, N. J. (1965): Ribonucleic acid synthesis in the rat uterus and its early response to estrogen. *Archives of Biochemistry and Biophysics*, 110, 284.
- Gorski, J. and Nicolette, J. A. (1963): Early estrogen effects on newly synthesized RNA and phospholipid in subcellular fractions of rat uteri. *Archives of Biochemistry and Biophysics*, 103, 418.
- Gorski, J., Noteboom, W. D., and Nicolette, J. A. (1965): Estrogen control of the synthesis of RNA and protein in the uterus. *Journal of Cellular and Comparative Physiology*, 66, (Supplement 1), 99.
- Greenman, D. L. and Kenney, F. T. (1964): Effects of alterations in hormonal status on ribosomes of rat uterus. *Archives of Biochemistry and Biophysics*, 107, 1.
- Hamilton, T. H., Widnell, C. C., and Tata, J. R. (1965): Sequential stimulations by oestrogen of nuclear RNA synthesis and DNA-dependent RNA polymerase activities in rat uterus. *Biochimica et Biophysica Acta*, 108, 168.
- Herranen, A. and Mueller, G. C. (1957): The effect of estradiol pretreatment on the serine aldolase activity of rat uteri. *Biochimica et Biophysica Acta*, 24, 223.

- Jensen, E. V. (1965): Metabolic fate of sex hormones in target tissues with regard to tissue specificity. Proc. Second International Congress of Endocrinology, London, 1964. Part II, p. 420, Excerpta Medica Foundation. Amsterdam.
- Jensen, E. V., De Sombre, E. R., and Jungblut, P. W. (1966): Interaction of estrogens with receptor sites in vivo and in vitro. Second International Congress on Hormonal Steroids (In Press).
- Jensen, E. V. and Jacobson, H. I. (1960): Fate of steroid estrogens in target tissues. p. 161, Biological Activities of Steroids in Relation to Cancer. Pincus, G. and Vollmer, E. P., eds. Academic Press, Inc., New York.
- Karlson, P. (1963): Chemie und Biochemie der Insektenhormone. Angewandte Chemie, 75, 257.
- Lippe, B. M. and Szego, C. M. (1965): Participation of adrenocortical hyperactivity in the suppressive effect of systemic actinomycin D on uterine stimulation by oestrogen. Nature, 207, 272.
- McCorquodale, D. J. and Mueller, G. C. (1958): Effect of estradiol on the level of amino acid activating enzymes in the rat uterus. Journal of Biological Chemistry, 232, 31.
- Moore, R. J. and Hamilton, T. H. (1964): Estrogen-induced formation of uterine ribosomes. Proceedings of the National Academy of Sciences. U. S., 52, 439.
- Mueller, G. C. (1953): Incorporation of glycine into protein by surviving uteri from estradiol treated rats. Journal of Biological Chemistry, 204, 77.
- Mueller, G. C. (1965): The role of RNA and protein synthesis in estrogen action, p. 228, Mechanisms of Hormone Action, A NATO Advanced Study Institute, Karlson, P. ed. Academic Press, Inc., New York.
- Mueller, G. C. (1966): Molecular Basis of Some Aspects of Mental Activity: A NATO Advanced Study Institute, Walaas, O. ed. Academic Press, Inc., New York.

- Mueller, G. C., Gorski, J., and Aizawa, Y. (1961): The role of protein synthesis in early estrogen action. Proceedings of the National Academy of Sciences, U. S., 47, 164.
- Mueller, G. C. and LeMahieu, M. A. (1967): Unpublished Data.
- Nicolette, J. A. and Mueller, G. C. (1966a): In vitro regulation of RNA polymerase in estrogen-treated uteri. Biochemical and Biophysical Research Communications, 24, 851.
- Nicolette, J. A. and Mueller, G. C. (1966b): The effect of actinomycin D on the estrogen response in uteri of adrenalectomized rats. Endocrinology, 79, 1162.
- Nicolette, J. A., LeMahieu, M. A. and Mueller, G. C. The in vitro regulation of estrogen-stimulated RNA polymerase from rat uteri. Submitted for publication.
- Noteboom, W. D. and Gorski, J. (1963): An early effect of estrogen on protein synthesis. Proceedings of the National Academy of Sciences, U. S., 50, 250.
- Noteboom, W. D. and Gorski, J. (1965): Stereospecific binding of estrogens in the rat uterus. Archives of Biochemistry and Biophysics, 111, 559.
- Notides, A. and Gorski, J. (1966): Estrogen-induced synthesis of a specific uterine protein. Proceedings of the National Academy of Sciences, U.S., 55, 1574.
- Summers, W. P. and Mueller, G. C. A study of factors regulating RNA synthesis in HeLa cells using MPB, a reversible inhibitor of RNA synthesis. Submitted for publication.
- Taiwar, G. P., Segal, S. J., Evans, A. and Davidson, O. W. (1964): The binding of estradiol in the rat uterus: A mechanism for depression of RNA synthesis. Proceedings of the National Academy of Sciences, U. S., 52, 1059.
- Tamaoki, T. and Mueller, G. C. (1965a): The effects of actinomycin D and puromycin on the formation of ribosomes in HeLa cells. Biochimica et Biophysica Acta, 108, 73.

- Tamaoki, T. and Mueller, G. C. (1965b): A rapidly labeled ribonucleoprotein in HeLa cell nuclei. *Biochimica et Biophysica Acta*, 108, 81.
- Toft, D. and Gorski, J. (1966): A receptor molecule for estrogens: Isolation from the rat uterus and preliminary characterization. *Proceedings of the National Academy of Sciences, U. S.*, 55, 1574.
- Ui, H. and Mueller, G. C. (1963): The role of RNA synthesis in early estrogen action. *Proceedings of the National Academy of Sciences, U. S.*, 50, 256.
- Vonderhaar, B. K. and Mueller, G. C. (1967): Unpublished Data.

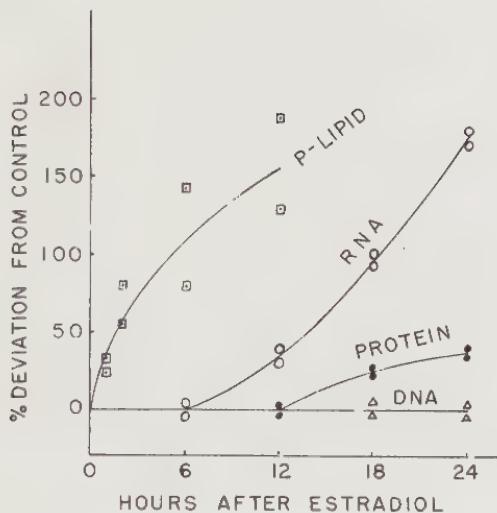


Figure 1. Alterations in the composition of rat uteri following a single dose of estradiol ( $10 \mu\text{g}$ ) injected at zero time. DNA was measured in  $\mu\text{moles}$  of thymine/uterus. RNA was measured as  $\mu\text{moles}$  of uridine and calculated as the ratio of uridine to thymine. Phospholipid was measured as  $\mu\text{moles}$  of ethanolamine phosphate. All data are expressed as the percent deviation from the control during the first 24 hours after hormone treatment.

Reprinted from Aizawa and Mueller (1961).

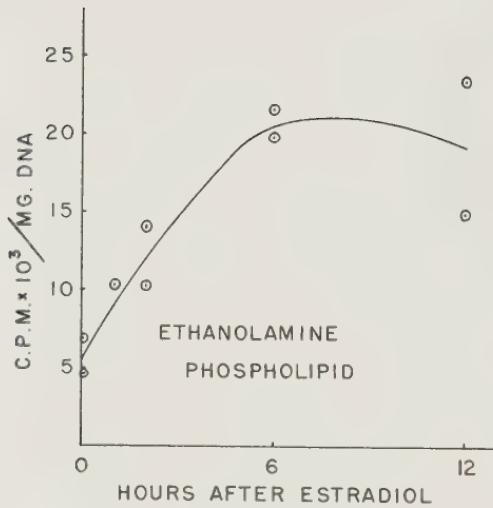


Figure 2. Effect of estradiol administration *in vivo* on the incorporation of inorganic orthophosphate- $P^{32}$  into ethanolamine phospholipids. Uterine segments from rats killed at varying times after a single injection of estradiol (10  $\mu$ g) were incubated with  $P^{32}$ . Data are expressed as c.p.m. in ethanolamine phospholipid fraction per mg. of DNA. Reprinted from Aizawa and Mueller (1961).

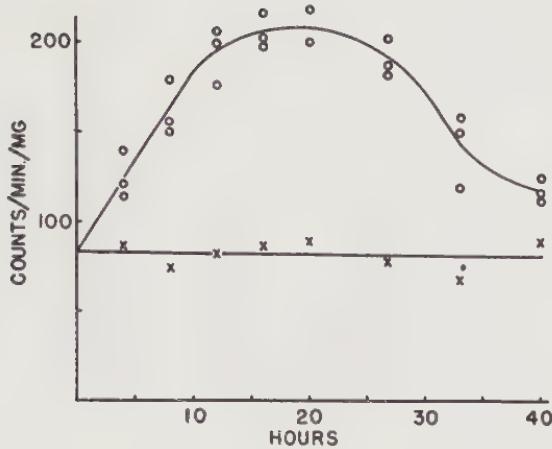


Figure 3. The effect of estradiol pretreatment on the rate of glycine-2-C<sup>14</sup> incorporation into surviving uterine segments incubated in vitro. Uterine segments came from rats pretreated for varying periods with estradiol (10 µg) in vivo. Reprinted from a paper by Mueller (1953).

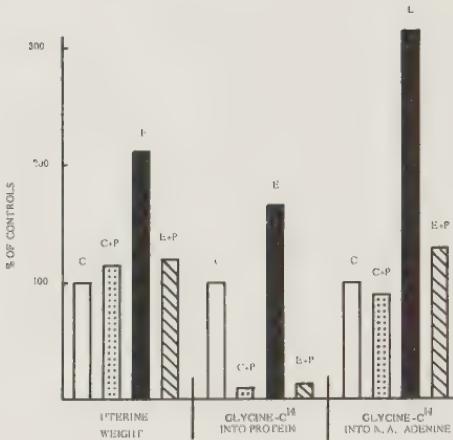


Figure 4. The prevention of the estrogen response by treatment with puromycin. Four hr before killing (0 hr) rats received 10  $\mu$ g estradiol (E) or a control solution (C). Puromycin (15 mg, P) was injected at 0, 1, 2, and 3 hr. All animals received glycine-2-C<sup>14</sup> at 0, 1, 2, and 3 hr. The uteri were analysed for wet weight, incorporation of glycine-2-C<sup>14</sup> into protein, and incorporation of glycine-2-C<sup>14</sup> into nucleic-acid adenine. The data are plotted as % of the control values. Data are taken from Mueller et al. (1961).



Figure 5. The effects of actinomycin D on the early response to estrogen. Groups of three rats were injected with 375 µg actinomycin D or a control solution 30 min. before receiving 10 µg of estradiol or a control solution. At 2 hours and 3 hours after the hormone treatment, uridine-H<sup>3</sup> (25 µC) and glycine-2-C<sup>14</sup> (6 µC) were injected. The rats were killed 4 hours after the hormone treatment, and the uteri were removed and analyzed for wet weight, counts/min. of H<sup>3</sup> into RNA, counts/min. of C<sup>14</sup> into protein and into mixed lipid fractions. The data are expressed as percent of the control values.

Reprinted from Ui and Mueller (1963).

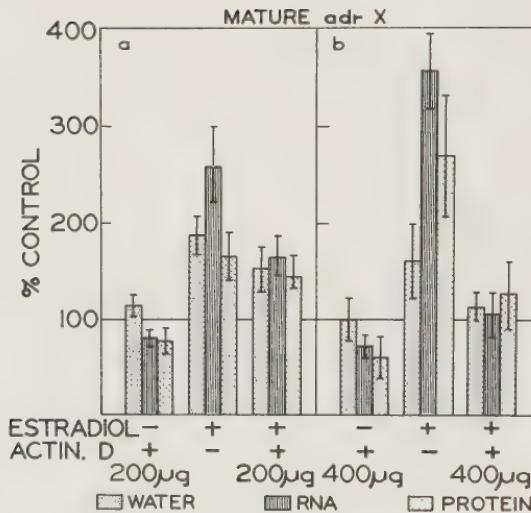


Figure 6. The effect of actinomycin D on the uterine response to 10  $\mu$ g estradiol in mature, ovariectomized, adrenalectomized rats. a. Rats received 200  $\mu$ g actinomycin D and 10  $\mu$ g estradiol as indicated. After 4 hours the uteri were removed from the animals and incubated in tissue culture medium with 1  $\mu$ C  $^{14}$ C-glycine. Each value is the average from 6 animals. Control values were: wet wt, 92.4 mg; RNA, 50 cpm/OD 260 m $\mu$ ; protein, 46 cpm/mg (gas flow count). b. Each rat was given 400  $\mu$ g actinomycin D; the incubation was with 5  $\mu$ C  $^{14}$ C-glycine. Each value is the average from 5 animals. Control values were: wet wt, 93.0 mg; RNA, 287 cpm/OD 260 m $\mu$ ; protein 783 cpm/mg (liquid scintillation count). Reprinted from Nicolette and Mueller (1966b).

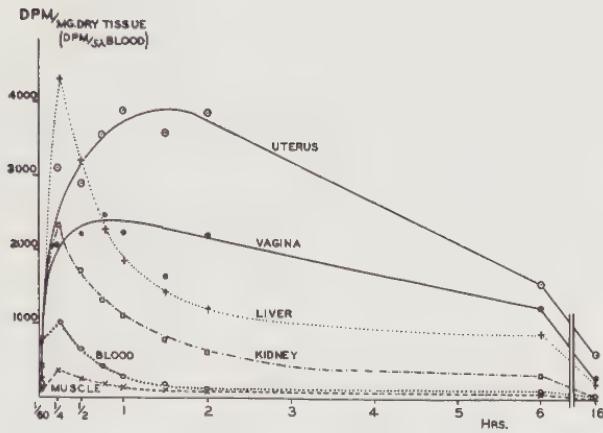


Figure 7. Concentration of radioactivity in rat tissues after a single, subcutaneous injection of estradiol ( $0.0098 \mu\text{g}$ ,  $117 \mu\text{C}/\mu\text{g}$ ). Liver and kidney points are mean values of three aliquots of dried, pooled tissue; other points are median values of individual samples from six animals (Jensen, 1960).

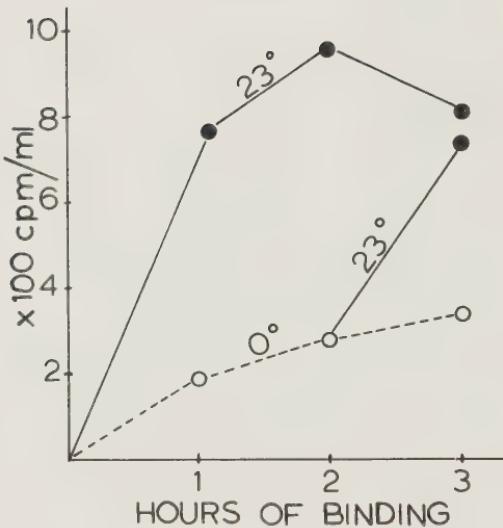


Figure 8. Effect of temperature on the binding of estradiol to receptor. Aliquots of receptor solution were incubated with estradiol 6, 7,  $\text{H}^3$  at  $0^\circ$  or  $23^\circ$  for the indicated times and passed through a biogel P-10 column to remove unbound estradiol. Bound estradiol is expressed as cpm per ml. of eluted receptor solution. (Vonderhaar and Mueller, 1967).

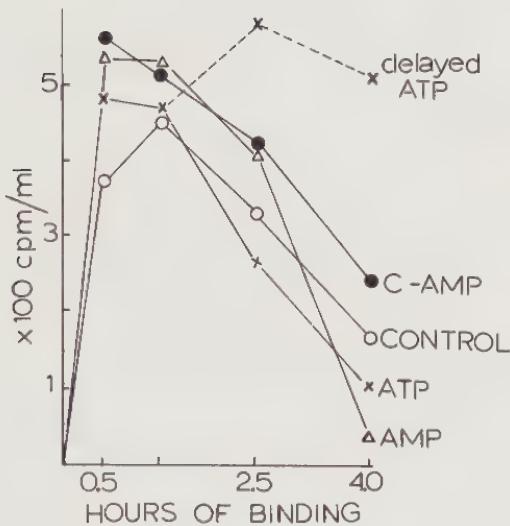
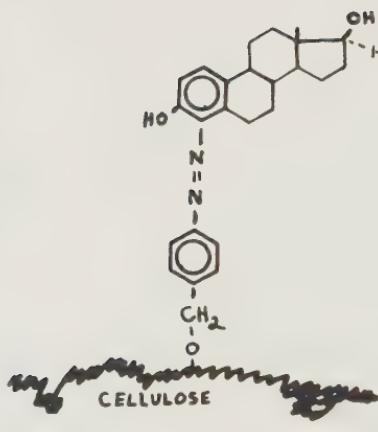


Figure 9. Effect of adenine nucleotides on the binding of estradiol to receptor. ATP, AMP, or 3,5 cyclic AMP at  $10^{-4} \text{ M}$  were added to the estradiol binding system at  $23^\circ$  where indicated and amount of bound estradiol determined as described in Figure 8. (Vonderhaar and Mueller, 1967).



PAB-ESTRADIOL

Figure 10. Basic structure of the PAB-estradiol cellulose resins. Diazotized p-aminobenzyl cellulose has been coupled to estradiol in the 2 or 4 position.

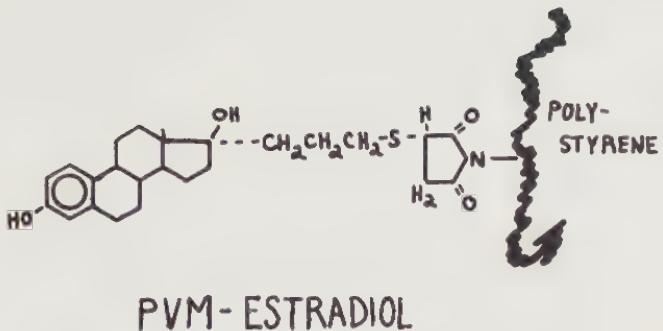


Figure 11. Basic structure of PVM-estradiol. The 17 $\alpha$  propyl mercaptan derivative of estradiol was coupled to a poly vinyl (N phenylenemaleimide resin) (Vonderhaar and Mueller, 1967).

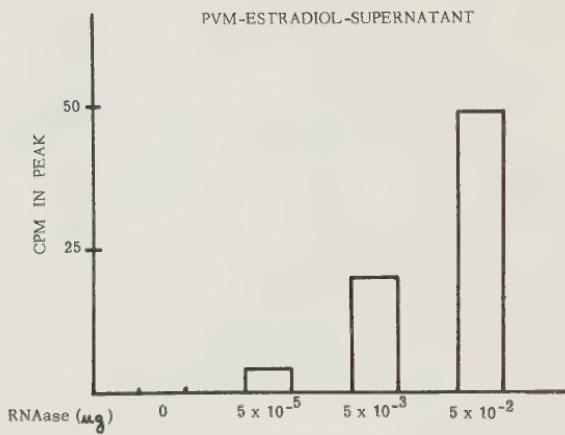


Figure 12. Effect of RNase on the binding of estradiol to receptor. Aliquots of receptor solution were incubated at  $0^{\circ}$  for 4 hours with PVM-estradiol resin and the filtrate exposed to estradiol- $\text{H}^3$  plus graded levels of RNase for 3.5 hrs. The amount of bound estradiol- $\text{H}^3$  was determined after passage through a biogel P-10 column. (Vonderhaar and Mueller, 1967).

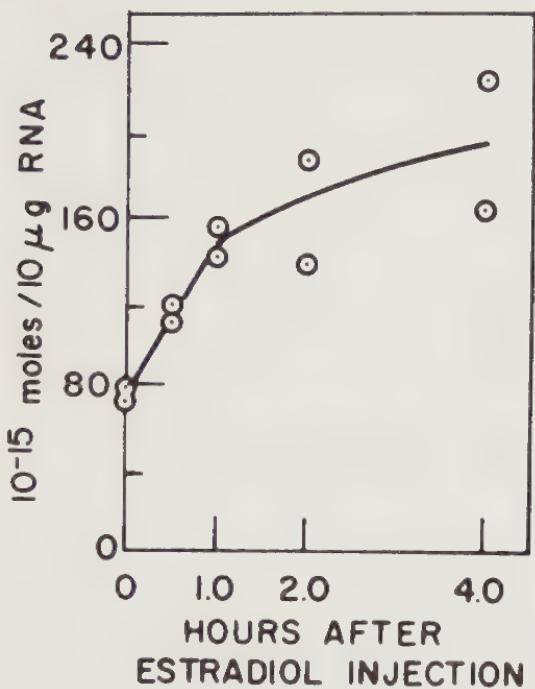


Figure 13. The stimulating effect of estradiol pretreatment on the level of RNA polymerase in rat uteri. Reprinted from a paper by Gorski (1964).

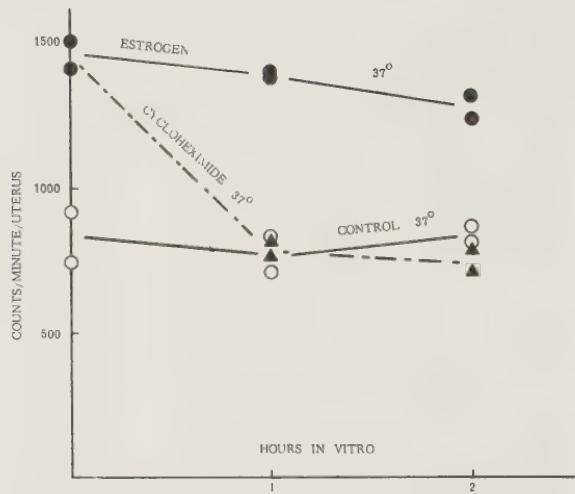


Figure 14. The stimulation of uterine RNA polymerase activity by estrogen (●—●) and the effect of cycloheximide (25 µg/ml) (▲—▲) on this activity. Estradiol (5 µg) given *in vivo* 4 hours before *in vitro* incubation at 37°C; controls (○—○) received no estradiol. RNA polymerase assayed by the method of Gorski (1964). Reprinted from Nicolette and Mueller (1966a).

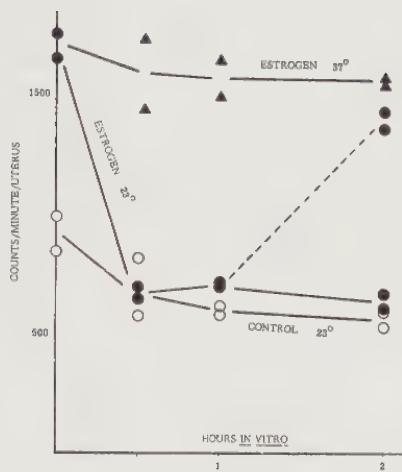


Figure 15. The effect of in vitro incubation of estrogen-treated (●—●) and control (○—○) uteri at 23° $C$  on uterine RNA polymerase activity. Polymerase activity of estrogen-treated uteri incubated continuously at 37° (▲—▲), or at 37° $C$  after 1 hour at 23° $C$  (●---●) were also determined. Reprinted from Nicolette and Mueller (1966a).

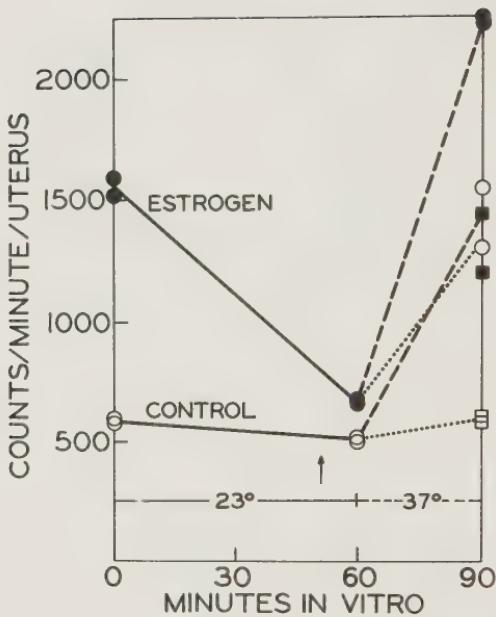


Figure 16. Effect of cycloheximide on the restoration of the temperature depressed RNA polymerase activity. Uteri from estrogen-treated (4 hours) and control rats were incubated at  $23^{\circ}\text{C}$  for 1 hour in vitro in tissue culture medium, then transferred to  $37^{\circ}\text{C}$  (dashed lines). Cycloheximide ( $25 \mu\text{g/ml}$ ) was added 10 min prior to transfer to  $37^{\circ}\text{C}$  (dotted lines). Nuclei were prepared at the indicated times and assayed for RNA polymerase by the incorporation of tritiated CTP into RNA. Nicolette and Mueller (1966).

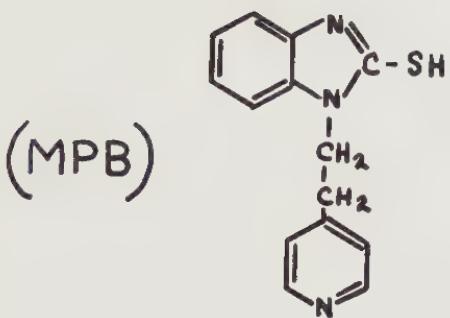


Figure 17. Structure of 2-mercaptopo-1-(β-4-pyridethyl) benzimidazole (MPB). An inhibitor of RNA synthesis in living cells.

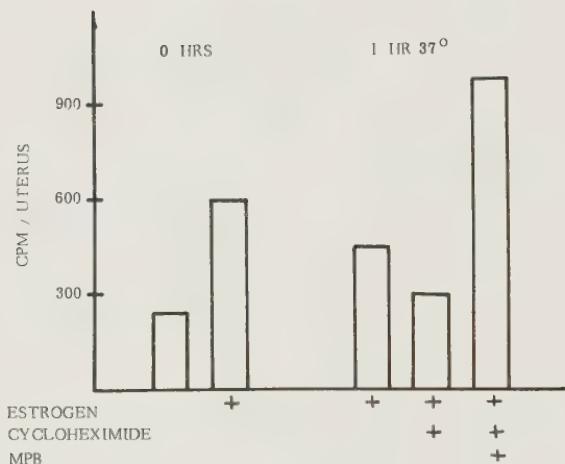


Figure 18. Reversal of the cycloheximide depression of RNA polymerase activity by MPB. Uteri from estrogen treated (4 hours) rats were incubated in tissue culture medium at  $37^{\circ}\text{C}$  for 1 hour; cycloheximide, 1  $\mu\text{g}/\text{ml}$  and MPB, 50  $\mu\text{g}/\text{ml}$  was added where indicated. Nuclei were then prepared from the uteri and assayed for polymerase activity and compared with the activity of tissues prior to incubation. RNA polymerase was measured by the incorporation of tritiated CTP into RNA. Mueller and LeMahieu (1967).

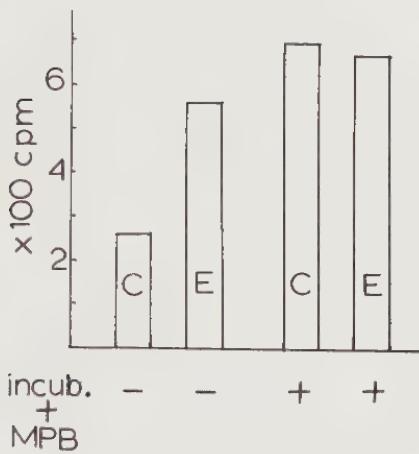


Figure 19. Effect of incubating surviving uteri with MPB on the level of RNA polymerase activity. Uteri from estrogen treated (4 hours) or control rats were assayed for RNA polymerase activity before and after incubation for 3 hours with MPB, 50 µg/ml and cycloheximide, 1 µg/ml at 37°C. RNA polymerase was measured in the nuclear fraction by the incorporation of tritiated CTP into RNA. Mueller and LeMahieu (1967).

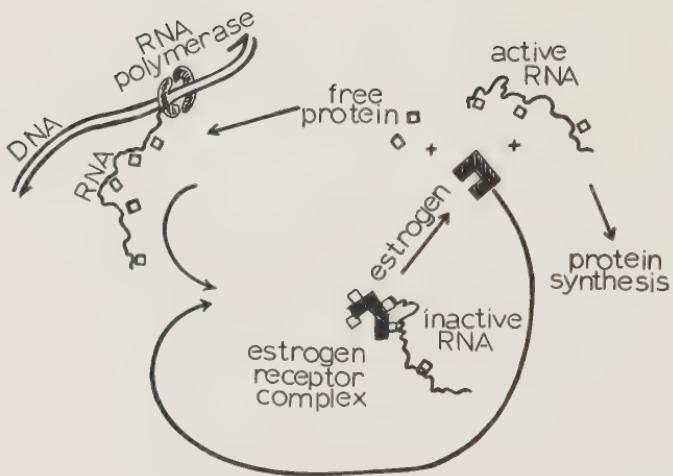


Figure 20. A hypothetical picture of estrogen action.