CONCLUSIONS

1). The following species of estrogen receptors have been purified to absolute homogeneity from the goat uterus. The homogeneity is demonstrated by the silver stained two dimensional gels (R-I, R-II and naER).
   a). Nuclear estrogen receptor R-I (Type-I)
   b). Nuclear estrogen receptor R-II (Type-II)
   c). Non-activated estrogen receptor (naER) from the cytosol
   d). Non-activated estrogen receptor (naER) from the plasma membrane

2). All of them are 66 kDa proteins, cross-react with rabbit anti-estrogen receptor (R-I) IgG and the polyclonal antibody against the bacterially expressed human estrogen receptor (hER). Monoclonal antibody against hER cross reacts only with the R-I (type I) estrogen receptor.

3). The R-II and the naER displayed identical CNBr peptide maps, distinctly different from the peptide map of the R-I receptor

4). naER forms a heterodimer with estrogen receptor activation factor (E-RAF), a DNA binding protein having no capacity to bind estradiol. E-RAF does not dimerize with R-I or R-II.

5). Plasma membrane appears to be the primary site of localization of the naER. Binding of estradiol is shown here to result in the dissociation of the naER-E2 complex from the plasma membrane into the medium under in vitro conditions. This naER dissociation from the plasma membrane is an estrogen-specific mechanism.
6). Unlike the R-I and the R-II estrogen receptors, the naER is shown here as a glycoprotein.

7). Both naER and R-II are tyrosine kinases. The tyrosine kinase activity is inhibited by Tyrphostin-25.

8). While the tyrosine kinase activity of the naER is totally inhibited by the presence of estradiol/ E-RAF in the assay medium, similar treatments have no effect on the tyrosine kinase activity of the R-II.

9). R-II binds to and phosphorylates specific subunits (91 kDa, 40 kDa and 20 kDa) of the goat uterine RNA polymerase II.

10). Deglycosylation of naER using a commercially available glycopeptidase resulted in the transformation of the naER to a R-II-like protein. It's affinity for binding estradiol reduced significantly as it's capacity to bind the hormone increased following the deglycosylation. The deglycosylated naER failed to dimerize with E-RAF. The values of Stokes radius and the sedimentation coefficient of the enzyme treated naER came very close to the corresponding values of the R-II. This apparently indicated that the deglycosylation brought about a total change in the shape and the molecular conformation of the naER, transforming it to a protein similar to the R-II.

11). The results are being summarized as follows: Estradiol binding to the naER in the plasma membrane is shown here to result in the dissociation of the naER into the cytosol along with two additional membrane proteins of molecular mass 55 kDa and 45 kDa. The functional significance of the latter two proteins remains unknown. The naER dimerizes with the E-RAF. A possibility is hereby indicated that a nuclear glycopeptidase
deglycosylates naER causing irreversible structural changes in the naER. I wish to speculate that the deglycosylated naER is the R-II estrogen receptor. The R-II binds to nuclear RNA polymerase II and phosphorylates three subunits of the enzyme. Even though it is possible to demonstrate the physical association of R-II ER with the nuclear RNA polymerase II, the mechanisms associated with the functional regulation of RNA polymerase II through phosphorylation of these three subunits remain unknown.

The term 'estrogen receptor' has been used with caution in order to identify both naER and the R-II. It has been realized that in a strict sense these two proteins do not have this qualification at this stage since the net result of the hormone binding to these proteins remains shrouded in mystery. This, however, does not dilute my confidence in that the real recognition of the R-II as an authentic nuclear estrogen receptor will be made sooner than later as there are experimental evidences, not presented in this volume, that substantiate this point. Confirmation of the precursor-product relationship between the naER and the R-II then will automatically qualify the naER to be identified as an estrogen receptor.