and progesterone was studied in rat and human uterus. The measurement of total (free and occupied) cytoplasmic and nuclear receptor was carried out by [^{3}H]-steroid exchange assays. Primary stimulation by estradiol in the rat uterus increased cytoplasmic and nuclear estradiol receptor (ERc and ERn) concentrations to about 10,400 sites/cell and 1260 sites/cell, respectively. However, administration of the above progestins brought about a dose dependent decline in the receptor concentration. The time course of changes in ERc indicated two phases of receptor replenishment, one between 3–9 h and second between 9–24 h. The second phase, which was partly dependent on protein synthesis, was sensitive to the inhibitory progestin block. Like ERc, progesterone receptor concentration (PRc) under the effect of progestins, decreased from an initial concentration of 8300 sites to 5100 sites/cell. Administration of norethindrone to women brought about a 50% decline in ERc and ERn levels. Similarly PRc levels in the proliferative phase of progestin treated women equalled those observed in mid secretory phase. Thus the modulation of uterine sensitivity to the hormones by limiting the receptor availability, appears to be one of the mechanisms by which progestins could exhibit the contraceptive effect at the uterine level.

96. Purification of sex hormone binding globulin by electrophoretic desorption from an affinity matrix

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A novel electrophoretic system for the purification of sex hormone binding globulin is described. The system utilises batch preparations of SHBG specifically immobilised on an affinity matrix (5α-androstane-3β,17β-diol-3β-hemisuccinate-Sepharose 4B) in a specialised small-scale electrophoretic cell. The electrophoretically desorbed protein was obtained in a purified and active form. The authors to date have achieved circa 1120 fold purifications using this single step procedure and succeeded in preparing 1.25 mg amounts of SHBG employing the cell in its present form. The application of the above principle to purification of a wide range of proteins using biospecific matrices together with results on the elution of glycoproteins from Concanavalin A-Sepharose, HSA from Cibacron Blue-Sepharose and steroid-specific antisera from steroid-Sepharose matrices are presented. The purified SHBG is characterised in terms of its molecular weight, electrophoretic mobility, amino-acid and carbohydrate composition.

6. MECHANISM OF ACTION

97. Oestradiol 2,4,6,7-[^{3}H] 17β uptake and subcellular distribution in the uterus of ovariectomized diabetic rat; induction of early protein synthesis

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Whether the metabolic alterations in the diabetic subject has a bearing on the regulatory mechanisms of oestrogenic action at the cellular level is largely unknown. The present study was designed to examine some of these parameters in ovariectomized, streptozotocin induced diabetic rats. At the conclusion of a 4 h infusion with 17β-oestradiol 2,4,6,7-^{3}H ($E_2{}^3H$) plasma samples and uteri were analyzed for total, free and conjugated radioactivity (R.A.). The subcellular distribution of R.A. in the uterus was analyzed on sucrose density gradients and the effect of oestrogen on early protein (I.P.) synthesis was studied. The results show that the uterine uptake of $E_2{}^3H$ in the controls and diabetic rats was not significantly different. The plasma however, showed a significantly higher level of total R.A. in the diabetic rats, due to the higher concentration of conjugated moiety. In the uterus, the subcellular distribution of R.A. did not show any major difference between the two groups. Sucrose density gradients of cytosol and Kcl soluble nuclear extracts showed similar peaks in both groups. Finally, the stimulation of I.P. synthesis gave identical responses, showing that the I.P. synthesizing potential was not modified in the diabetics. In conclusion, streptozotocin induced diabetes of short duration (24 h–6 weeks), involving high glycemia but minor ketoacidosis did not modify the subcellular binding nor the hormonal activity of oestradiol in the rat uterus.

98. Physico-chemical characteristics of oestradiol and oestrone binding to macromolecules in the fetal uterus of guinea pig

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Specific binding of oestradiol (E_2) and oestrone (E_1) were evaluated in fetal uteri throughout fetal development of the guinea pig. The values are similar for these two estrogens and increase with fetal development. After incubation of the cytosol fraction with 4.1×10^{-9} M [^{3}H]-E_2 or [^{3}H]-E_1, the specific binding of [^{3}H]-E_2 is (average of 5 experiments): 85 fmol/mg protein at 36–37 days of gestation, 390 at 44–45 days; 410 at 49–50 days; 720 at 60–66 days and 600 in newborns (3–4 days). For [^{3}H]-E_1 these values are, respectively, 74, 270, 350, 550 and 530. The K_D for [^{3}H]-E_2 is $2–4 \times 10^{-10}$ M and for [^{3}H]-E_1 $8–9 \times 10^{-10}$ M. Specific binding sites are also found in the nuclei after incubation of the total fetal uterine cell with [^{3}H]-E_1. Qualitative analysis of the radioactive material which was specifically bound to macromolecules shows that in the [^{3}H]-E_2 incubation 80–85% of the radioactivity remained as non metabolized E_2; similarly, for the incubation of [^{3}H]-E_1, 90–95% is non metabolized E_1. Oestrone competes with similar intensity for the formation of [^{3}H]-E_2 complexes and vice-versa, estradiol competes with the [^{3}H]-E_1 complex. It is concluded: (1) that specific uterine binding sites for E_2 and E_1 are present during intrauterine life, (2) that these specific binding sites increase during fetal development, (3) that the sites of binding are the same, and (4) that the conversion of oestrone ⇄ oestradiol is very limited in this fetal tissue.

99. Estrogen receptor: nuclear retention and uterotrophic activity of Centchroman; a comparison with estradiol-17β

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The temporal profile of estrogen receptor binding by the rat uterine nuclei (determined by exchange assay) and uterotrophic response following a single pharmacological dose of estradiol-17β (E_2), (2.5 or 10 μg/rat, s.c.) and Centchroman (C), (25 or 100 μg/rat, s.c.), a nonsteroidal estrogen possessing post-coital contraceptive activity, was examined. Both the doses of C caused prolonged elevation in the nuclear receptor (Rn) levels. A good correlation was found between the Rn levels and the temporal pattern of uterine response, the high dose giving a relatively more