boys the mean SHBG concentration dropped from 7.7 mg/l seen at pubertal stage I to 3.1 mg/l at pubertal stage V. A decline, although not so steep as seen among the boys, was also noted in sexually maturing girls.

91. Androgen receptor in the bursa of Fabricius

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By glycerol gradient ultracentrifugation analyses an "8S" radioactive peak could be demonstrated in the cytosol of the bursa of Fabricius from 12-day-old chicken embryos after labelling with [^{3}H]-androstanolone 1 nM. An excess of 100 nM unlabelled androstanolone completely suppressed the radioactive peak, while the inhibition was important but not complete with 100 nM unlabelled cyproterone acetate. Previous heating of the cytosol at 37 C for 40 min completely prevented the binding of radioactive androstanolone. With another technique, Sephadex G-25 chromatography and after labelling the cytosol with [^{3}H]-androstanolone or [^{3}H]-testosterone the presence of a radioactive excluded fraction which was suppressed by an excess of unlabelled testosterone was shown. So by two different techniques a high affinity, saturable, "8S", macromolecular component which had the different characteristics of a classical androgen cytosol receptor could be demonstrated in the cytosol of the bursa of Fabricius of 12-day-old chicken embryos. 17β-estradiol, androstenedione and progesterone inhibited the binding of androgens but diethylstibestrol, cortisol and dexamethasone did not. An identical androgen receptor was found to be present in the cytosol of the bursa of Fabricius from quail embryos. The bursa of 12-day-old chicken embryos contained 70 fmol/mg of protein and 420 fmol/mg of nuclear DNA. Dissociation experiments of the epithelium from endodermal origin and the mesenchymal part of the bursa showed that the number of receptor sites was greater in the epithelium. In other tissues of the same embryos such as lung and small intestine the number of binding sites was much lower (4 fmol and 2.5 fmol/mg of protein). These experiments are in favour of a direct mode of action of androgens on this lymphopoietic organ and could possibly explain the inhibition of the development of the bursa when androgens are injected in the incubated egg and also the role of those hormones in the normal involution of this organ.

92. 5α-Dihydrotestosterone (5α-DHT)-specific binding proteins in the plasma and reproductive tract of the male goat

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Previous studies have shown that the seminal plasma of the adult goat contains a 5α-DHT-specific binding protein. The aim of this study was to determine if this specific binding protein has, like most of the proteins in seminal plasma in many species, a plasmatic origin (via the accessory glands) or a testicular origin (via the epididymis) or both. This work was carried out on adult goats using the polyacrylamide gel electrophoresis method. Preliminary investigations demonstrated the presence of a sex steroid binding protein (SBP) with a R_F of 0.3, in the blood plasma. In addition, the presence of a specific binding protein (R_F = 0.3) in the seminal plasma of such animals was confirmed. Furthermore, the presence of androgen binding protein (ABP) with the same R_F was also demonstrated in the cauda epididymal plasma. As SBP and ABP appeared to have the same R_F, we looked for a specific binding protein in accessory gland plasma of vasectomised goats and seminal plasma of cowperectomised, vesiculectomised goats. Under these conditions, no specific binding protein was found in the seminal fluid of vasectomised animals, whereas an ABP (R_F = 0.3) has been demonstrated in the seminal plasma of animals without cowper's glands and seminal vesicles. Consequently, the specific binding protein identified in the seminal plasma has a testicular origin via the epididymis.

93. Specificity and cross-reactivity of primate sex steroid binding plasma protein (SBP)

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The presence of SBP in several vertebrate species has been confirmed for several species and detected in many others, by measurement of specific [^{3}H]-dihydrotestosterone ([^{3}H]-DHT) binding in plasma. Cross-reactivity between the monospecific anti-human SBP anti-serum and plasma from different species studied by immunoelectrophoresis and immunodiffusion occurred only with primate plasma. Moreover, total identity is obtained only between man and Pongidae (chimpanzee) whereas partial indentity is observed with other monkeys.

The similarities between human and monkey SBP were also evident in terms of steroid binding specificity: DHT > 5α-androstane-diols > testosterone > estradiol. However the affinity of estradiol and estrone increased from man to new world monkeys. (Estrone is not bound by human SBP.)

It would appear that human and monkey SBP display a number of common antigenic determinants which increase progressively in the evolutionary scale, from prosimii to old world monkeys, becoming identical between chimpanzee and man.

94. Effects of progesterone on D-amino acid oxidase *in vivo* and *in vitro* studies

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Progesterone has been shown to inhibit purified hog kidney D-amino acid oxidase (DAAO). In rat kidney homogenates progesterone was found to have two effects on DAAO activity—an inhibitory effect in the absence of FAD and either a stimulatory effect or no effect in the presence of added FAD. Apo-DAAO prepared by charcoal treatment of crude homogenate could be activated by *in vitro* addition of FAD. Progesterone did not inhibit this activation. Data suggest different effects of progesterone on DAAO, apo and holo enzymes. Ovariectomy did not produce a change in the kidney DAAO, although in the liver the enzyme activity showed a slight tendency to decrease. Ovariectomy led to a greater *in vitro* inhibition of kidney DAAO by progesterone. Intraperitoneal progesterone injection (10 mg/kg body weight) to ovariectomised animals reversed this effect.

95. Progestin mediated modulation of steroid hormone receptors

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The influence of progestins like norethindrone and norethindrone acetate on receptor concentrations for estradiol

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