sol and nuclei was investigated. Kinetic analysis of cytosol revealed the presence of specific binding sites with a K_D of 11×10^{-9} M and a receptor site concentration of 0.32 pmol/ng protein. The complex was labile at a temperature of 45 C. The specificity was indicated in a competition study using unlabelled competing steroids at 10, 100, 1000-times the molar concentration of radioactive hormone. The inhibition was similar for both cold testosterone and DHT (80%), methyltestosterone (66%), androstenediol (64%), DHA (54%), androstenedione (42%), E_2 (40%), E_3 (3%). The specific binding protein has a sedimentation coefficient of 5S. Purified nuclei or whole tissue incubated with [^{3}H]-T or [^{3}H]-DHT at 35 C for 1 h gives a nuclear radioactivity that is completely inhibited by cold competitor and with approximately the same nuclear binding sites/DNA respectively for [^{3}H]-T and [^{3}H]-DHT. Our data support the presence of an androgen receptor in human placental cytosol the role of which needs further investigation.

77. Estrogen receptors in lactating mammary gland of the rat

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Kinetic and molecular properties of estradiol receptor (ER) in cytosol were characterized by titration analyses, DNA-cellulose binding, ion-exchange chromatography and density gradient centrifugation. The rate constants for association and dissociation at 0 C were $2–3 \times 10^{-7}$ M^{-1} min^{-1} and $2–4 \times 10^{-3}$ min^{-1}, respectively. These data and those of Scatchard analyses indicated binding sites with high affinity ($K_D = 10^{-10}$ M). Only estrogenic compounds with $^{c}3$ and $^{c}17\beta$ hydroxyl functions, as well as unsaturation of A ring, were bound. The 8S ER complex chromatographed as 2 components on DEAE-cellulose columns. ER binding to DNA–cellulose was increased significantly if charged receptor complex was warmed at 28 C for 30 min; whereas the presence of 1 mM EDTA during activation reduced binding to DNA-cellulose by 40–50%, an effect that can be reversed by divalent cations. Activation of charged ER was a prerequisite for the translocation of ER into nuclei. Activated ER stimulated Mg^{2+} dependent RNA polymerase activity 3-fold without altering Mn^{2+} dependent activity. These results support the notion that activation of steroid receptors is essential for their translocation into nuclei and subsequent stimulation of nuclear synthetic activity.

78. Androgen and progesterone binding in human testis cytosol

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The present report describes an attempt to detect, characterize and measure in human testis cytosol proteins corresponding to the androgen receptor which has been found to be present in the testis of hypophysectomized rats. The methods used have been exchange assay with [^{3}H]-methyltrienolone ([^{3}H]-R 1881), studies of dissociation rates and heat sensitivity, as well as sucrose gradient centrifugation. In normal human testis cytosol, a concentration of 119 ± 59 fmol/mg protein receptor-like androgen-binding activity, was found. In only two out of five tumour specimens (seminomas) a relatively low (23–33 fmol/mg protein) exchange activity could be detected. Receptor affinities for R 1881 and testosterone were found to be about equally high, whereas the affinity for progesterone was slightly lower. There was only negligible binding inhibition by oestradiol. Gradient centrifugation revealed two peaks, corresponding to 3S and 4S, respectively. Progesterone affinity could be demonstrated solely for the 4S peak. The results indicate that two types of high affinity binding proteins are present in human testicular cytosol, one with affinity for both androgens and progestins, and the other with specific androgen-binding activity.

79. Estrogen induction and functional importance of carrier proteins for riboflavin and thiamine in the rat during gestation

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During gestation in higher animals thiamine (B_1) and riboflavin (B_2) are preferentially transported across the placental barrier and concentrated by the foetus. The mechanisms of such facilitated transport are unknown. We have shown in the hen that vitamin transport for the embryonic development is mediated through B_1 and B_2 carrier proteins. We report here that in the pregnant rat (but not in the male or immature female) similar proteins with immunological cross reactivities to chicken B_1 and B_2 carrier proteins do exist. The hormone responsible for induction of these proteins was estrogen (E) since (1) specific induction of these proteins in the male and in ovariectomized adult female rats could be elicited by E (2) the blood levels of these proteins alter in concert with the changing E in cycling female and pregnant rats. Passive immunization of pregnant rats (4–16 d) with antibodies to chicken vitamin carrier proteins (but not to ovalbumin) resulted in foetal resorption/abortion showing functional importance of carrier proteins for embryonic development and survival.

80. Influence of prolactin on testosterone production and action in the male rat

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Prolactin (PRL) facilitates testosterone (T) action on male accessory sex organs of rodents, but the mechanism is not clear. A temporal relationship between circulatory levels of PRL and T in developing rats was observed. In 90-day old rats, neutralization (for 5 days) of PRL by specific antiserum (A/S) significantly reduced the serum T and weights of ventral prostate and seminal vesicles, whereas injection of PRL (1 mg/100 g.b.wt. × 5d) to such rats significantly enhanced serum T levels and the weights of the accessory organs. However, their testes responded to saturating levels of LH to a similar extent in terms of T production *in vitro*. Injection of PRL enhanced the ability of the prostate and seminal vesicles to concentrate [^{3}H]-T *in vitro*, whilst A/S treatment had no significant influence. PRL treatment enhanced the binding of [^{125}I]-LH to testicular membrane preparations whilst A/S was without any effect. Neither PRL nor A/S modulated the binding of [^{125}I]-PRL to membranes.

81. Protein induction and estrogenic potency

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Estrogen treatment has become more frequent during the last years. There are few methods to quantify and compare the estrogenic effect of various preparations. Animal

models, gonadotrophin-inhibition and receptor affinity studies have been used to describe the biological effect of estrogen. The use of inducible plasma proteins in comparison has several advantages. Changes in concentration reflect the "efferent" expression of steroid influence. Factors like intestinal absorbtion protein-binding, receptor affinity and intracellular metabolism are included in the net result of an increased protein synthesis. The estrogenic effect can be directly followed and quantified in patients sera. An estrogen inducible plasma protein (PZP) was used in a study of estrogenic potency. A total of 211 women were followed before and during treatment with 11 different estrogenic preparations. The serum concentration of PZP after an initial induction phase reached a stable plateau level. The plateau level was taken as a parameter for estrogenic potency. An estrogen index for the different preparations was constructed.

82. Steroid hormone binding to cytoplasmic receptors: additivity of the relative binding affinity (RBA) increments calculated for individual substituents

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A close examination of steroid conformations has revealed that, apart from a few exceptions, they are conditioned by a handful of basic structural elements which once assembled are subject to little further modification. If the zones of a receptor which are involved in binding possess only moderate adaptability, this structural stability of the steroid molecule might be reflected in additivity of binding properties and consequently of parameters such as relative binding affinities (RBA). The RBA is a measure of the displacing power of one ligand with respect to another (usually the endogenous ligand) in relation to a particular receptor. By comparing the RBAs of pairs of molecules differing by the same substituent for 5 different steroid hormone receptors (estrogen (ES), progestin (PG), androgen (AND), mineralo- (MIN) and gluco-corticoid (GLU)), mean RBA increments were deduced for this substituent for each receptor. On the basis of these data an approximation of the RBA of any molecule could be calculated by adding the RBA increments corresponding to all its substituents. A comparison of these calculated RBAs with measured RBAs revealed a good correlation for the PG, AND, MIN and GLU receptors, which bind primarily 3-keto 4ene steroids, and for the ES receptor, which binds nearly exclusively compounds with a phenol A ring. This remarkably predictive, yet very simple, method based on additivity gives a first approximation of the RBA of a molecule which could be further improved by deducing RBA increments by comparing steroids differing by more than one substituent—by computer rather than manual analysis.

83. Lack of receptor binding specificity of steroids related to their conformational mobility

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Unsaturated Δ4.9 and Δ4.9.11 steroid hormones with a keto group in position 3 and a hydroxy group or lactonic (or spirosultine) ring in position 17β, with or without methyl substituents in position 17α,18 and/or 7α, tend to compete significantly for specific binding to several steroid hormone receptor proteins (progestin (PG), androgen (AND), mineralo- (MIN) and gluco-corticoid (GLU)). If the interaction between ligand and receptor is compared to a lock and key fit, the presence of several common structural features among ligands binding to different receptor proteins suggests a close affiliation among these proteins. The ability of any one ligand to bind effectively to more than one receptor implies a degree of conformational adaptability exceeding that of the natural hormones. The molecular flexibility and mobility of several Δ4,9 and Δ4,9,11 unsaturated steroids was established by X-ray crystallography and by molecular geometry calculations, e.g. R 2323 (13-ethyl-17-hydroxy-18,19-dinor-17α-pregna-4,9,11-trien-20-yn-3-one) which binds to the PG, AND and GLU receptors, has 5 independent molecular conformations with a total flexibility covering 3.3 Å; between-conformation transition energy is less than 2 kcals and therefore far less than that necessary for binding to these receptors ($K_D \sim 10^{-9}$ M). On the basis of these data on unsaturated derivatives and also on other substituents affecting receptor binding specificity, the regions of interplay (hydrogen bonds, van der Waals forces) between a ligand and the receptor corresponding to a particular hormone class have been defined.

84. Heterogeneity of glucocorticoid binding sites: a classical and a unique binder in bovine tissues

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Bovine tissues were found to possess two separate cytoplasmic binding sites for glucocorticoids. The first, (GR) appeared to be a classical glucocorticoid receptor: high affinity for [^{3}H]-triamcinolone acetonide (TA) ($K_D \sim 5$ nM), low capacity (240 fmol/mg protein), and the usual sequence of steroid specificities TA > dexamethasone (DEX) > B > PROG > T = E_2. The second, (X) also had a high affinity for [^{3}H]-DEX ($K_D \sim 10$ nM), but had a higher capacity (400 fmol/mg), and failed to bind TA (DEX > PROG > B > T = E_2 = TA). In addition, X was uniquely stable to treatments which destroyed GR: heat, pronase and trypsin. Both GR and X were present in several bovine organs including thymus, liver, adrenal cortex, and adipose tissue; neither was present in plasma. [^{3}H]-TA and [^{3}H]-DEX entered the nucleus. Unlabelled TA blocked [^{3}H]-DEX transfer, indicating all nuclear uptake was via GR. Further proof that GR was the true receptor was obtained in thymocyte function studies where TA was as potent as DEX in inhibiting [^{3}H]-uridine incorporation. In conclusion, GR resembles glucocorticoid receptors in other species. X is heat and protease resistant, and discriminates between TA and DEX. The function of this unique site is yet to be determined.

85. Endometrial membrane–steroid hormone interaction: fluorescence probe analysis

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Studies on the effects of steroid hormones on the endometrial mitochondria show that there is a qualitative alteration in the membrane when it transforms from the proliferative to the secretory phase. Interaction of fluorescence probe, ANS, with the mitochondria is affected by steroids. In the proliferative phase K_a is high but in the secretory phase, K_a is very low, keeping limiting fluorescence unaffected. *In vitro* effects of steroid hormones and their derivatives show that progesterone increases the limiting fluorescence without changing K_a whilst oestrogen, medroxyprogesterone acetate and norethisterone acetate increase K_a of ANS interaction, and limiting fluorescence is slightly