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

altered. Effects of various membrane-active drugs on the fluorescence of mitochondria-bound ANS shows that, with the alteration in hormonal status of the endometrium, the drug-binding pattern changes and binding is generally strong at the secretory phase and weak at the proliferative phase. In order to find out the probable mechanism of steroid induced alteration in mitochondrial drug binding, lipid distribution patterns of mitochondrial membranes have been analysed. It is concluded that ovarian steroids, by altering lipid microenvironment, change drug and ANS binding pattern.

**86. Binding of ethynyloestradiol-3-sulphate to human plasma proteins**

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The binding of ethynyloestradiol-3-sulphate (EE-3-S) to plasma proteins has been investigated, as within 1 h of administration most of the EE found in the circulation is present as EE-3-S. [$^3$H]-EE-3-S and unlabelled EE-3-S were synthesised using a sulphur trioxide-triethylamine complex. Gel filtration of a plasma sample incubated at 37°C with [$^3$H]-EE-3-S suggested that albumin was the main protein binding EE-3-S. Equilibrium dialysis experiments showed that EE-3-S associates with human serum albumin (HSA) to the same extent as with total plasma proteins. HSA has two sets of binding sites for EE-3-S, $n_1 = 1$ and $n_2 = 9$ with association constants of about $10^6$ and $10^4$ $M^{-1}$ respectively. Natural oestrogen sulphates but not unconjugated oestrogens displaced EE-3-S from the strong binding component of HSA. From this study it is concluded that EE-3-S, like the natural oestrogen sulphates, circulates bound to albumin.

**87. Interaction of chlormadinone acetate with the proteins of human plasma**

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The binding of chlormadinone acetate (CAP) to proteins of human plasma was investigated in detail as one of the possibilities to explain its prolonged half-life and low metabolic clearance rate in women. These studies revealed that the CAP binds to proteins in the human plasma. The CAP binding plasma protein has a Stokes radius of 33 Å, molecular weight of 56.000 and sediments at an $S$ value of 4.1. The binding of CAP to plasma proteins was maximum at pH 7–8 and the equilibrium of CAP binding was achieved after 48 h. The plasma protein bound CAP with an intrinsic association constant at $2.6 \times 10^6$ $M^{-1}$ and had $15 \times 10^{-9}$ mol/mg binding sites. Estradiol and progesterone were found to be able to displace CAP from its binding sites to plasma proteins whereas testosterone was inactive in this respect. The physicochemical properties of the CAP binding plasma proteins and the competitive displacement studies with natural sex hormones, revealed that the CAP binds to specific proteins in the plasma with a high affinity and low capacity. The CAP-binding plasma protein was found to be other than serum albumin.

**88. A-ring conformation and receptor protein binding**

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Analysis of conformational data on 285 estranes, androstanes and pregnanes collected in the first two volumes of the *Atlas of Steroid Structure* allows a characterization of the nature of skeletal and side chain flexibility, substituent influence and hydrogen bond directionality. Substituent effects alter the electronic and conformational features of flexible points in the steroid. The observations that 4ene-3-one A-ring conformation can be inverted by (1) C(9)-C(10) unsaturation, (2) 19-methyl removal, (3) configurational change at C(9) and C(10), or (4) combined 17α-acetoxy and 6α-methyl substitution and that steroids with high binding affinity for the progestin receptor have these structural features has led to the proposal that high affinity binding is primarily due to A ring–protein interactions. Evidence from other structural studies is consistent with an A-ring-initiated binding model. We propose that the remainder of the steroid may contribute to conformational change in the receptor or genome interaction. (Research supported by Grant No. CA-10906 from the National Cancer Institute, DHEW.)

**89. Androgen-binding protein in rat liver cytosol**

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A study was made of the nature of a macromolecular component in the hepatic cytosol of male rats which has affinity binding with testosterone and androstenedione. The high association rate and the low dissociation rate of this component with testosterone and androstenedione indicated the specificity of the binding. This component was inactivated by incubation with proteolytic enzymes and by heating, but was unaffected following incubation with RNase. Analysis by sucrose density gradient centrifugation revealed that this component had a sedimentation coefficient of 10S. Assuming from the elution profile of both of the components which bind testosterone and androstenedione on gel chromatography, the molecular weight and the similarity of the structure of these androgens, it is likely that there is one form of the binding protein for both testosterone and androstenedione. It was postulated that the only difference in binding for these androgens is that testosterone, which has a more potent catabolic action, showed a more rapid association rate with the protein.

**90. An optimized saturation assay for SHBG in children during their multiphase sexual maturation**

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Sex-hormone-binding globulin (SHBG) is a consistuent of human blood which binds the principal androgens and oestrogens with high affinity. The saturation assay for SHBG originally described by Rosner was modified in order to optimize assay conditions. Quantitation of SHBG was accomplished by replacing centrifugation with filtration which allowed 10-fold higher steroid excess than used in the original method and reduced the statistical error. Further, an extrapolation method was developed by which SHBG concentrations greater than the limit set by steroid concentration may be corrected for 100% saturation. In