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 2.6×10^6 M^{-1} and had 15×10^{-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