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

REED, M. J.* and FOTHERBY, K., Department of Steroid Biochemistry, Royal Postgraduate Medical School, London, W.12, England (*Present address Department of Chemical Pathology, St. Mary's Hospital Medical School, London, W2, England)

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

DUGWEKAR, Y. G.*, Department of Reproductive Biology, All India Institute of Medical Sciences, New Delhi, India; *Present Address: Department of Obstetrics & Gynaecology, C.O.V.S., Punjab Agricultural University, Ludhiana, India

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

DUAX, W. L., GRIFFIN, J. F., ROHRER, D. C. and WEEKS, C. M., Medical Foundation of Buffalo, Inc., 73 High St., Buffalo, NY 14203, U.S.A.

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

OTA, M., SATO, N. and OBARA, K., Department of Biochemistry, Iwate Medical University School of Medicine, Morioka, Japan

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

BLANK, B., SCHEEL, H., RAGER, K. and GUPTA, D., Department of Diagnostic Endocrinology Universitäts-Kinderklinik, 7400 Tübingen, Federal Republic of Germany

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

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

MICHEL, G. H. and BAULIEU, E. E., Department of Endocrinology, Centre Hospitalier de Luxembourg and U.33 INSERM Bicêtre, France

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

JEGOU, B. and CHEMINEAU, Ph., I.N.R.A., Station de Physiologie de la Reproduction 37380 Nouzilly, France

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)

RENOIR, J. M., BAULIEU, E. E. and MERCIER-BODARD, C., Laboratory of Hormones, 94270 Bicêtre, France

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

AHMED, F. and BAMJI, M. S., National Institute of Nutrition, Hyderabad, India

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

KASID, A. and LAUMAS, K. R., Department of Reproductive Biology, All India Institute of Medical Sciences, New Delhi, India

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