but highly significant ($P < 0.005$) time related change in the non-specific binding of $E_2$ by plasma and a significant ($P < 0.05$) change in plasma unbound $E_2$ measured by ED-RIA were observed.

**51. Measurement of unbound testosterone and oestradiol levels in human plasma,** PATRICIA J. LOBO, C. E. HORTH and R. F. PALMER, Department of Metabolic Studies, G. D. Searle & Co. Ltd., High Wycombe, Bucks, U.K.

Data from conventional techniques for measuring the unbound concentrations of sex-hormones in plasma may tend to underestimate their availability to the intracellular environment where the steroids are bound by high affinity cytosolic receptor proteins. A combined equilibrium dialysis (ED) radioimmunometric assay (RIA) technique involving the presence of a mixed preparation of antibodies to testosterone (T) and oestradiol ($E_2$) in the buffer compartment of the dialysis cell was devised in order to measure unbound T and $E_2$ direcly. Forty samples of plasma could be processed simultaneously by one operator. The within-assay coefficients of variation (CV%) were $\pm 2.88\%$ ($n = 10$) and $\pm 4.12\%$ ($n = 10$) for T and $E_2$ respectively. Between assay CV% was found to be $\pm 9.65\%$ ($n = 10$) and $\pm 20.11\%$ ($n = 10$) for T and $E_2$ respectively. The theoretical values for the unbound concentrations of each steroid showed a high degree of association with their measured concentrations over a wide range with correlation coefficients ($r$) of 0.998 and 0.967 for T and $E_2$ respectively. Canrenone (SC-9376) and potassium canrenoate (SC-14266), the principal metabolites of the steroidal agent spironolactone, were found not to significantly interfere in the assay. In studies of plasma from normal subjects the unbound concentrations of T were found to be $2.01 \text{ ng ml}^{-1} \pm 0.39$ (S.D.) in men and $0.48 \text{ ng ml}^{-1} \pm 0.21$ (S.D.) in women. The unbound concentrations of $E_2$ were $0.012 \text{ ng ml}^{-1} \pm 0.004$ (S.D.) in men and $0.071 \text{ ng ml}^{-1} \pm 0.027$ (S.D.) in women. It was concluded that the technique would be of use in monitoring the levels of unbound T and $E_2$ in men and women with pathological conditions or on long term medication.

**52. Biospecific affinity chromatography of microbial steroid transforming enzymes,** P. ATRAT, V. DEPPMEYER, C. HÖRHOLD and K. SCHUBERT, Academy of Sciences of the GDR, Research Centre of Molecular Biology and Medicine, Central Institute of Microbiology and Experimental Therapy, Department of Steroid Biochemistry, Jena, German Democratic Republic

4-En-3-oxosteroid(acceptor)-1-en-oxidoreductase (steroid-1-dehydrogenase) from *Nocardia opaca* and 4-en-3-oxosteroid-5α-reductase from *Mycobacterium smegmatis* were highly purified by biospecific adsorption. Thus the steroid-1-dehydrogenase was enriched in one step of affinity chromatography 100 fold and 600 fold in combination of this method with chromatography on aminoalkylsepharose [1]. The 5α-reductase was obtained in a 33 fold purification by affinity chromatography [2]. The affinants on sepharose basis have been proved to be the best. A testosterone ligand was bound to the matrix via a spacer of about 20 Å. These affinants were prepared by coupling of *N*-(4-androstene-3-on-17β-oxycarbonyl)-ε-amino caproic acid with aminohexylsepharose 4B or adipinic acid dihydrazidoyl-sepharose 4B respectively, yielding a ligand density of 4 μmol/ml. The nature of the biospecific binding has been studied by the method of affinity chromatography. In this way the hydrophobic character of the binding has been detected [3]. By addition of water soluble organic solvents with different hydrophobicities a linear correlation between matrix-binding of the enzyme and the hydrophobicity of the solvents was found using the log P values of Hansch [4]. Thus the influence of organic solvents to affinity chromatography can be described quantitatively. Further the affinity chromatography was used nearly quantitative for separation of the cofactor (FAD) of both enzymes from immobilized enzyme–ligand complexes (pH 3, 1 M $(NH_4)_2$ $SO_4$) [5,6]. By this easy and quick technique the apo-enzyme can be obtained in a highly purified state. The binding strength of the apoenzyme to the immobilized steroid ligand is highly decreased in comparison to the native enzyme and can be interpreted by the action of a rest hydrophobicity. That indicates the essential character of FAD for both ligand binding and transformation.

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**53. Prolactin and adrenal androgen secretion,** S. ANDO and A. VERMEULEN, Section of Endocrinology and Metabolic Diseases, Academic Hospital, University of Ghent, Ghent, Belgium

The aim of this study was to investigate the eventual role of prolactin in the secretion by the adrenal of dehydroepiandrosterone (D) and its sulphate (DS). Therefore prolactin and D(S) levels were determined in different groups of subjects under various physiopathological conditions. In patients with prolactinoma, or with pharmacologically induced hyperprolactinemia, plasma DS and, to lesser extent, D levels were elevated, whereas the DS blood production rate was greatly increased. ACTH stimulation which did not influence DS levels in normals, caused a significant increase in patients with prolactinoma; the increase in D levels was similar in normals and in prolactinoma patients. Bromocriptine treatment of prolactinoma patients induced a normalisation of both prolactin and D(S) levels.

Acute elevation of prolactin levels in normal subjects by TRF stimulation or by short term administration of sulpiride, on the contrary to long term treatment, did not influence D(S) levels however and during pregnancy, notwithstanding high prolactin levels, D(S) levels were lower than during the menstrual cycle. In patients with prolactinoma, treated with cortisol in substitutive dose, D(S) levels were low (normal) notwithstanding persisting high prolactin levels.

It is concluded that only prolonged elevation of prolactin levels induces increased D(S) secretion by the adrenal cortex and that a normal ACTH secretion is a prerequisite for this effect. The absence of elevated DS levels in pregnancy might be explained by an intense metabolism, as the DS production rate is increased. The intimate mechanism of prolactin and ACTH interaction at the adrenal cortex remains, however, unknown.

**24A. Induction of maturation in *Xenopus laevis* oocytes by a steroid linked to a polymer,** J. FRANÇOIS GODEAU*, SABINE SCHORDERET-SLATKINE†, PATRICK HUBERT‡ and ETIENNE-EMILE BAULIEU*, *Lab. Hormones, 94270 Bicêtre, France, †Zoologie Experimentale, Université de Genève, Switzerland, ‡Chimie Physique Macromoléculaire Ensic, 54000 Nancy, France