apparent association constants were 0.9 and $0.6 \times 10^8\ M^{-1}$ at the 12th and 16th days respectively. For aldosterone two classes of binding sites were evidenced; one with high affinity and low capacity and another with low affinity. The number of high affinity binding sites for aldosterone were 37, 68 and 74 fmol/mg protein at the 12th, 16th and 21st days respectively. The apparent association constant decreased progressively from the 12th day to the 21st day of embryogenesis (from 4.3 to $2.5 \times 10^8\ M^{-1}$). The peak of corticosterone receptor concentration occurs at a time of enhanced adrenocortical enzymes activities [1] thus suggesting a relationship between the adrenal hormonogenesis and the ontogeny of corticosterone receptors in kidneys of chick embryo.

Reference

1. Lehoux J.-G.: *Molec. cell Endocr.* **2** (1974) 43.

32. Radio-receptor-assay of plasma steroids, C. BONNE, M. MOUREN, M. M. BOUTON, D. PHILIBERT and J. P. RAYNAUD, Centre de Recherches Roussel-Uclaf, 93230 Romainville, France

A quick, simple method for measuring the plasma hormones (free and/or bound) specific to a particular hormonal class has been developed. Whereas radioimmunoassay can measure the concentration of a single hormone (disregarding cross-reactivity), it cannot measure all the steroids corresponding to a hormonal class, especially if the structure of these steroids is unknown. In the radio-receptor-assay, hormonal class specificity is achieved by basing the method upon the interaction of the hormones present in the plasma with specific tissue receptors. These hormones are measured by displacement of a synthetic radioligand which binds to a single tissue receptor only and which does not bind to plasma proteins. Thus, for the determination of endogenous androgens, progestins and estrogens in plasma, cytosol samples from rat prostate, rabbit uterus (from estrogen-primed rabbits) and mouse uterus were incubated with plasma in the presence of the following synthetic radioligands, respectively: [^{3}H]-R 1881 (17β-hydroxy-17α-methyl-estra-4,9,11-trien-3-one), [^{3}H]-R 5020 (17,21-dimethyl-19-nor-4,9-pregnadiene-3,20-dione) and [^{3}H]-R 2858 (11β-methoxy-17-ethynyl-1,3,5(10)-estratriene-3,17β-diol). Specific binding was measured by a Dextran-coated charcoal adsorption technique as a function of plasma dilution and the total endogenous hormone concentration was expressed in terms of the reference radioligand used for the assay. In each case, testosterone, progesterone and estradiol were measured in parallel by a specific radioimmunoassay (without chromatography) with BSA-coupled antigens obtained with the following derivatives: 11α-hemisuccinate of testosterone (sensitivity = 10 pg); 7-carboxymethyloxime of estradiol (10 pg) and 11α-terephthalate of progesterone (20 pg). Possible applications of the radio-receptor-assay of plasma are: (a) evaluation of the repercussions of the administration of a drug on steroid hormone profiles in clinical pharmacology studies, (b) the study of changes in these profiles in pathological hormonal imbalance (e.g. acne, menopause) and (c) the detection of anabolic agents (doping).

33. Progesterone receptor induction and RNA polymerase activation by estrogen derivatives, M. M. BOUTON and J. P. RAYNAUD, Centre de Recherches Roussel Uclaf, 93230 Romainville, France

The two ethynyl estradiol derivatives, R 2858 (Moxestrol = 11β-methoxy ethynyl estradiol) and RU 16117 (11α-methoxy ethynyl estradiol) differ structurally by the configuration of the methoxy substituent in position 11. They form complexes with the cytoplasmic estrogen receptor in mouse uterus at the same rate, but the RU 16117–receptor complex dissociates three times faster than the estradiol receptor complex and about 20 times faster than the R 2858–receptor complex at 0°C. This difference in kinetics explains their unequal ability to maintain estrogen action. (a) The time-course of cytoplasmic progestin binding site induction (from 6 h to 7 days) was measured in the uterus by an exchange technique with tritiated R 5020 (17,21-dimethyl-19-nor-4,9-pregnadiene-3,20-dione) following priming of castrated mice with 1 μg of estrogen derivative. R 2858 was able to maintain high progestin binding site concentrations longer than estradiol and much longer than RU 16117. (b) Uterine nuclear RNA polymerase A and B activities were measured 6 and 24 h after estrogen derivative administration to immature mice. RU 16117 activated RNA polymerases A and B to the same extent as R 2858 and estradiol at 6 h, but unlike these two compounds no longer had any effect at 24 h. (c) Uterine weight was measured 6, 24 and 48 h after estrogen administration. An increase in weight was recorded with both R 2858 and estradiol over this time; with RU 16117, uterine weight had returned to the control level at 48 h.

34. Protein binding and extraction of plasma steroids for clinical assays, R. MONDINA, P. CAPETTA, P. CREMONESI, E. GROSSI and F. POLVANI, II[a] Clinica Ostetrico-Ginecologica dell'Università, Milano Laboratorio di Chimica degli Ormoni del C.N.R., Milano, Italy

The evaluation of plasma steroids is generally carried out after a preliminary extraction step. Steroids in plasma are bound by multiple ligand systems i.e. SHBG, transcortin, albumin and probably others, but until now the theoretical aspect of extraction from plasma has not been completely investigated. Extraction for assay of steroids is generally carried out by liquid–liquid (plasma–organic solvent) system and the recovery is usually evaluated by the added labelled hormone. In this work we studied the recovery with different organic solvents and at different incubation times of labelled hormone (testosterone) from plasma of pregnant women, normally menstruating women, normal men and from seminal plasma.

Results

(1) In fresh blood and seminal plasma incubated at room temperature with labelled testosterone, the binding increases rapidly but decreases significantly before equilibrium is reached (after 100 min). In frozen plasma this behaviour is generally modified, and very inconstant. (2) At equilibrium the plasma–organic solvent partition coefficient is not strictly equal to the values given for water–organic solvent and varies between the cases.

Conclusions

The evaluation of extraction recovery by labelled hormone requires an incubation of at least 100 min since before the plasma steroid binding presents a rapid paradox behaviour. At the present time the significance of this observation and perspectives in the clinical field are under investigation.

35. Progesterone binding plasma proteins of pregnancy in hystricomorph rodents, NICOLA ACKLAND, R. B. HEAP and BARBARA J. WEIR, Agricultural Research Council Institute of Animal Physiology, Babraham, Cambridge, CB2 4AT, England

Progesterone binding globulin (PBG) was first identified in pregnant guinea-pigs as a plasma protein with a high