14. Estrogen receptor: inhibition of aggregation and dissociation into subunits by chemical perturbation, V. SICA, E. NOLA, G. PONE, G. A. PUCA and F. BRESCIANI, Instituto di Patologia generale, 1[a] Facoltà di Medicina, Università di Napoli, Naples, Italy

A generally recognized problem in dealing with estrogen receptors, and with steroid hormone receptors at large, is the high tendency of these proteins to aggregate following tissue homogenization. By means of chemical perturbation, we have now succeeded in inhibiting such aggregation and even favor dissociation of receptor into subunits, as shown by (1) absence of large aggregates and indefinite inhibition of their formation in crude uterine low-salt cytosol; no aggregates form even during application of concentration procedures like salting out by $(NH_4)_2SO_4$; (2) sharp peaks of estrogen receptor activity on Sephadex chromatography of crude uterine cytosol in low-salt; the peaks are "included" into Sephadex G-100; (3) appearance of a smaller estrogen binding unit with the following properties: Svedbergs, 2·8; Stokes radius, 28 Å; mol. wt., 32,000; frictional ratio, 1·44. Past and present data are compatible with the hypothesis that the 32,000 mol. wt. species is a subunit which may self-associate to form larger structures. This specific self-association is distinct from aggregation of receptor with other macromolecules of cytosol, a process which is started by homogenization of the tissue and progresses with aging of cytosol. Inhibion of aggregation by chemical perturbation removes an important obstacle in the way of purification of receptor in tangible amounts. (Research supported by the Consiglio Nazionale delle Ricerche, Rome.)

15. Analytical study of gluco- and mineralocorticoid receptors in rat kidney. Dramatic protective effect of ligand on mineralocorticoid receptors, M. E. RAFESTIN-OBLIN, A. MICHAUD, J. MENARD and P. CORVOL, INSERM U 36, 17 rue du Fer-à-Moulin, 75005 Paris, France

Rat kidney cytosol contains both gluco (GC) and mineralo (MC) corticoid receptors labelled respectively by [^{3}H]-dexamethasone (^{3}H-DM) and [^{3}H]-aldosterone (^{3}H-A). The purpose of this work was to compare the stability of these receptors in presence or in absence of their ligand. Three different experimental procedures were used: (a) Kidney slices experiments: kidney slices from adrenalectomized rats were incubated 20 min at 20° with ^{3}H-A or ^{3}H-DM in presence or in absence of competitors. Slices were homogenized at 4° in 0·25 M sucrose, 1 mM $MgCl_2$ and the cytosol was then prepared. (b) Delayed *in vitro* incubation: kidneys were homogenized in 0·01 M Tris–HCl, 1 mM EDTA, pH 7·4, containing 10% glycerol. The homogenate was centrifuged at $700\,g \times 10$ min and the supernatant centrifuged at $105000\,g \times 1$ h was used for the binding studies. (c) Direct *in vitro* incubation (protected cytosol): the homogenate was centrifuged at $700g \times 10$ min and the supernatant was immediately incubated with ^{3}H-DM or ^{3}H-A. Cytosol was then prepared as above. Correction for unspecific binding was systematically performed by adding a 1000-fold excess of non-radioactive steroid. When kidney slices were incubated with 5×10^{-10}M ^{3}H-A. A inhibited the binding of ^{3}H-A to its cytosol receptor 8 times more effectively than DM. However, with receptors recovered in the cytosol fraction, A was 2 times less effective than DM in competing for ^{3}H-A binding sites. This was confirmed by a series of experiments: (1) when cytosol receptors were protected during preparation by adding steroids after homogenization, A was again the best competitor for ^{3}H-A binding, (2) stability studies showed that after 1 h at 0° unfilled MC receptors were completely lost whereas 50% of the unfilled GC receptors were still present after 5 h. About 30% of A-filled receptors could be detected after 10 h at 0° whereas 80% of DM-filled receptors were recovered, (3) Scatchard plot of ^{3}H-A showed two slopes on protected cytosol, the steeper slop ($Kd_1 = 6 \times 10^{-9}$M) corresponding to the MC receptor. In unprotected cytosol only the lower slope corresponding to GC receptors ($Kd_2 = 8 \times 10^{-8}$M) was detected. In conclusion: (1) GC and MC binding sites are rapidly destroyed in kidney cytosol unless they are protected by their ligand, (2) GC sites are much more stable than MC sites, (3) it is impossible to study MC receptors in complete *in vitro* experiments in kidney cytosol without protecting them after homogenization.

16. Androgen receptor assay with a specific ligand, [^{3}H]-methyltrienolone C. BONNE and J. P. RAYNAUD, Centre de Recherches Roussel-Uclaf, 93230 Romainville, France

A new methodology for the measurement of androgen binding sites has been developed on animal models with a view to its subsequent application to normal and pathological human tissues. This methodology meets two basic requirements: firstly, the need to distinguish between tissue and plasma binding and secondly, the need to take binding of the endogenous hormone into account. Although binding to the intracellular receptor and to sex steroid plasma binding protein (SBP) may be distinguished by separating the proteins, by agar-gel electrophoresis for instance, the number of binding sites involved can only be estimated with difficulty by these techniques. The use of the synthetic androgen, methyltrienolone (R 1881), labelled with high specific activity (58·2 Ci/mmol), as a radioligand, in a Dextran-coated charcoal method, precludes the necessity of protein separation since this compound is not bound by human plasma (i.e. SBP) but is bound with a higher affinity than androstanolone (DHT) by intracellular receptors ($1/Ka_{R\,1881} = 0{\cdot}6 \pm 0{\cdot}1$ nM, $1/Ka_{DHT} = 1{\cdot}4 \pm 0{\cdot}2$ nM on rat prostate cytosol). The total number of binding sites is determined by exchanging the bound endogenous hormone with radioligand under conditions where the natural hormone–receptor complex is dissociated, but where neither loss of binding sites nor ligand metabolism occur. These conditions are fulfilled by the use of tritiated methyltrienolone since, unlike androstanolone, methyltrienolone is not metabolized by the 3-ketosteroid hydrogenases in cytosol.

17. A comparative study of the biological activity and affinity to cytosol receptor of rabbit uterus of optical, structural and isomeric analogs of estradiol, S. N. ANANCHENKO, T. I. BARKOVA, G. A. CHERNYAEV, V. V. EGOROVA, G. D. MATARADZE, V. B. ROSEN, N. A. SOKOLOVA and I. B. SOROKINA, Shemyakin Institute of Bioorganic Chemistry, U.S.S.R. Academy of Sciences and Laboratory of Endocrinology, Lomonosov State University Moscow, U.S.S.R.

Competitive binding between analogs of estradiol with estradiol receptors of cytosol from rabbit uterus has been studied. The following compounds have been used: D-estradiol-17β and its optical isomers (D,L-,L-estradiol-17β); D-3-deoxyestradiol-17β; D-17-deoxyestradiol; D-3,17-bis-deoxyestradiol; D,L-8-isoestradiol-17β; D,L-D-homoestradiol-17$a\beta$; D,L-3-deoxy-D-homoestradiol-17$a\beta$; D,L-8-iso-D-homoestradiol-17$a\beta$; D-estrone. The Allen–Doisy assay