tion to be used as a receptor. The experiment consisted in comparing the binding value of the receptor treated with anti-estrogens or synthetic estrogens. As a result, it was clear that 16β-ethyl estradiol inhibited the binding of [6,7-^{3}H]-estradiol-17β with receptor protein and also U-11,100A, U-11,555A, clomiphene and epithioandrostanol inhibited the binding in some degree. Synthetic estrogens except methallenestril inhibited the binding. While estrone, estriol, testosterone and progesterone have no competitive action with [6,7-^{3}H]-estradiol-17β in the binding with receptor protein.

23. **High affinity binding of glucocorticoids, estrogens and androgens in cytosols of human mammary carcinomas,** F. A. G. TEULINGS, R. E. TREURNIET, J. ALEXIEVA-FIGUSCH, J. BLONK-VAN DER WIJST and H. A. VAN GILSE, Rotterdamsch Radio-Therapeutisch Instituut, Rotterdam-3024, The Netherlands

Soft tissue metastases of patients with advanced mammary carcinomas often respond well to therapy with glucocorticoids. It is not known whether the presence of high affinity binding proteins for glucocorticoids is essential for a response and it has also not been established whether the presence and concentration of glucocorticoid "receptors" in tumor tissue is related to the estrogen and androgen receptor concentrations or to the age of the patients. Low temperature agar gel electrophoresis was used for the quantification of high affinity binding of glucocorticoids in cytosols (tissue–buffer 1:2, w/v). [^{3}H]-dexamethasone was used as the radioligand. Simultaneously estrogen and androgen receptor concentrations were measured using [^{3}H]-estradiol-17β and [^{3}H-5α]-dihydrotestosterone. The ranges (and median values) of the receptor concentrations (pM/l) have been compared between patients under and over 55 years of age:

A slight positive correlation seems to exist between the respective receptors in the group of 21 cytosols: the partial correlation coefficients (Spearman-rank) are: estrogen–androgen 0·50; estrogen–glucocorticoid 0·01; androgen–glucocorticoid 0·43.

In conclusion, high affinity binding for glucocorticoids in mammary carcinomas can be found in low concentrations, independent of the age of the patients and not clearly related to the presence of androgen or estrogen receptors.

24. **Human renal carcinoma and steroid hormone receptors,** G. CONCOLINO, A. MAROCCHI, M. L. MARTELLI, V. GAGLIARDI and F. DI SILVERIO, Instituto di Patologia Speciale Medica e Metodologia Clinica II, and Clinica Urologica, University of Rome, Italy

In previous investigations using agar-gel electrophoresis (Wagner) we demonstrated the presence of estradiol and progesterone receptors in normal human kidney. The study is now extended to six human renal adenocarcinoma. Cytosols (200,000 *g* supernatant) or cytosols treated with a suspension of charcoal in buffer for 18 h were incubated *in vitro* at 0° 1–4 × 10^{-9}M tritiated estradiol and progesterone; the tritiated steroid bound to protein was determined by electrophoresis at low temperature. With this technique estradiol receptor and the estradiol-SHBG complexes were easily discriminated. Presaturation of CBG with cortisol (1 × 10^{-5}M) was necessary to ascertain the presence of progesterone receptors. The specificity of the binding was verified by adding a 100-fold excess of non-radioactive steroids. The presence of a specific receptor for progesterone was demonstrated in all the tumours examined. On the contrary estradiol receptor was found only in three out of the six carcinoma studied. Scatchard plot analysis was done in order to measure the binding capacity and the dissociation constant. Progesterone, which is known to produce a regression of these tumours in animals and has been used for the treatment of renal adenocarcinoma also in the human, in our experience does not compete with estradiol at the receptor level.

25. **Translocation of specific steroid hormone receptors into purified nuclei *in vitro* in Syrian hamster tissues and estrogen-dependent renal tumor,** JONATHAN J. LI and SARA ANTONIA LI, Department of Medicine, SDTU, Veterans Administration Hospital, 55417, Department of Pharmacology, Medical School, University of Minnesota, Minneapolis, Minn., 55455, and Department of Biological Chemistry, LHRRB, Harvard Medical School, Boston, Massachusetts, 02115, U.S.A.

The estrogen-induced and dependent renal tumor in the Syrian hamster is a unique steroid responsive tissue in that specific cytosol estrogen (8S, 8S+4S), progesterone (6–7S), and androgen (8S) receptors reside in the same tissue. The presence of these receptors was detected by incubation of the tumor cytosol at 0°C with 2–5 × 10^{-9}M tritiated steroid *in vitro* and subsequent sucrose gradient analyses after Dextran-charcoal treatment. Receptor specificity was assessed using a competitive binding assay with various steroid metabolites and anti-steroidal agents. The steroid receptors in the renal tumor have properties similar to those found in the hamster uterus and seminal vesicles, respectively. To elucidate the requirements for steroid hormone receptor translocation, we examined the ability of the renal tumor steroid receptors and both hamster and rat uterine receptors to translocate *in vitro* into target and non-target nuclei of hamster tissues. Incubation of uterine cytosols from hamster and rat and renal tumor with purified nuclei (determined by electron microscopy) from hamster renal tumor, kidney, and liver at 28°C for 20 min resulted in nuclear [^{3}H]-estradiol-5S receptor complex in all tissues in 0·4 M KCl gradients. The magnitude of nuclear retention was renal tumor ⩾livers >kidney based on equivalent DNA content. The amount of cytosol translocated into nuclear preparations also depended on the cytosol receptor used, hamster uterus ⩾renal tumor >rat uterus. [^{3}H]-Dihydrotestosterone binding in the renal tumor cytosol translocated into nuclei of all tissues examined and nuclear extracts contained a 3·2S receptor which is clearly distinguishable from that of the nuclear estrogen receptor complex. However, hamster uterine and renal tumor progesterone receptors did not translocate into non-target nuclei. (Supported by National Cancer Institute Grant CA 16854-01 and Research Service, Veterans Administration Hospital, Minn.)

26. **Comparison of the binding of [2,4,6,7-^{3}H]-estradiol-17β (^{3}H-E_2) to the nucleus in the immature rat uterus under *in vivo*, *in vitro* and cell-free conditions,** E. EKKA and R. DE HERTOGH, Endocrinology and Nutrition Unit, Hôpital St. Pierre, University of Louvain, Leuven, Belgium

In order to establish if any direct and close relationship existed between the experimentally induced status of the cytosol receptor and its binding to the nuclear component(s), the following study was undertaken. Crude nuclear pellets, from uteri of immature Wistar R rats (28 days old), after *in vivo* infusion of 180 ng/h of ^{3}H-E_2 for 4 h, or after *in vitro* incubation of whole uteri at 0–4°C

or at 22°C in the presence of 3×10^{-9}M ^{3}H-E_2: or after cell-free incubation at 0–4°C of nuclei with cytosol pre-incubated with ^{3}H-E_2 at 0–4°C or at 22°C, were extracted with Tris–HCl buffer containing 0·3 M KCl at pH 8·5. The respective KCl soluble nuclear extracts were analyzed on 5–20% sucrose density gradients containing 0·3 M KCl, after treatment with Dextran-coated charcoal (250 mg % charcoal + 25 mg % Dextran). The sedimentation profiles were compared with respective profiles of the corresponding cytosol estradiol–receptor complex. The data obtained show that under cell-free conditions conducted at 0–4°C, a major "4S" peak and a shoulder in the "5S" region were present both in the cytosol and the nuclear extracts. Cytosol pre-incubated at 22°C and the nuclear extract of nuclei incubated with this cytosol showed that the "5S" peak became more important than the "4S" peak in both the cytosol and the nuclear extracts. Under *in vitro* conditions, uteri incubated at 0–4°C showed mainly a "4S" peak and a minor "5S" peak in the cytosol; the nuclear extract, on the contrary, showed a major "5S" peak and a shoulder in the "4S" region. On incubation at 22°C the "4S" peak in the cytosol had a reduced but similar profile; whereas the "5S" peak in the nuclear extract became more important. Under *in vivo* conditions, the cytosol was characterized by a single peak in the "4S" region and the nuclear extract by a "5S" peak. The nuclear extracts on treatment with charcoal showed comparable sensitivity under the three experimental conditions. Chase experiments *in vivo* had shown earlier that bound estradiol in cytosol and nucleus was exchangeable. Similarly addition of excess unlabeled estradiol under *in vitro* and cell-free conditions resulted in a disappearance of the bound ^{3}H-E_2 in the "4S" and "5S" form both in the cytosol and the nuclear extracts. It can therefore be argued that although cell-free and *in vitro* nuclear bindings differ from that observed *in vivo*, nevertheless, activation of the cytosol receptor by temperature promotes conformational modification of the receptor and enhances binding to the nuclear component(s) in a form which approaches that observed under physiological conditions of *in vivo* infusion of the hormone.

27. **Androgen binding in rat uterus cytosol,** W. HEYNS, G. VERHOEVEN and P. DE MOOR, Regal Instituut, Minderbroedersstraat 10, B-3000 Leuven, Belgium

After 6 h of infusion of [^{3}H]-testosterone(T) to adult rats the ratio of [^{3}H]-5α-dihydrotestosterone (DHT) to [^{3}H] – T was 18·1 in the prostate and 0·014 in the uterus. This observation, which confirmed the presence of a DHT-"receptor" in the prostate and suggested the presence of a "T"-receptor in the adult rat uterus, as described for immature rats (Giannopoulos, 1973), prompted us to study the specificity of the "receptor"-proteins of both organs. When comparing testosterone binding in uterus and prostate cytosol, similar values were obtained for the concentration (66 vs 43 fmol/mg) and the apparent K_d (1·1 vs 1·2 nM) of the binding sites. Although the binding of DHT appeared to be weaker in the uterus, the competitive effect of more than 20 other steroids on T binding in uterus cytosol and on T or DHT binding in prostate cytosol was similar. Several arguments suggest that the binding of DHT in the uterus is only apparently weaker than the binding of T. Indeed, the dissociation of bound DHT under chase conditions was much slower than the dissociation of T. Furthermore, DHT was very intensively metabolized during incubation at 0°C with formation of 5α-androstane-3α,17β-diol, a weakly-bound component. Finally, after adequate pretreatment of the uterus cytosol, the binding of DHT increased markedly and exceeded the binding of T. From these results and from other data such as their precipitability by ammonium sulfate or by protamine sulfate and their behaviour during gel filtration and ultracentrifugation it is concluded that the androgen "receptors" from uterus and prostate show no marked difference and may be identical.

28. **The presence of α-fetoprotein in the 8S macromolecular complex of rat uterine cytosols,** J. URIEL, D. BOUILLON, C. AUSSEL and M. DUPIERS, Institut de Recherches sur le Cancer, B.P. n° 8, 94800 Villejuif, France

Alpha-fetoprotein (AFP) is the first α-globulin to appear in mammalian serum during development and the dominant serum protein in early embryonic life. The estrogen binding activity of rat, mouse and human AFP has been previously demonstrated by immunological methods (J. Uriel, B. de Néchaud and M. Dupiers, *Biochem. biophys. Res. Commun.* **46** (1972) 1175). The identity between serum AFP and the 4–5S macromolecular complex of uterine cytosols from immature rats (10–23 day old) has been recently reported (C. Aussel, J. Uriel, G. Michel and E. E. Baulieu, *Biochimie* **56** (1974) 567; G. Michel, E. E. Baulieu, C. Aussel and J. Uriel, *Steroids* **24** (1975) 437). We present here data which provided evidence that the 8S macromolecular complex formed at low salt concentration in these cytosols is also made up of AFP, probably in combination with other(s) macromolecular constituent. AFP appears to account mainly, if not entirely, for the high affinity estrogen binding properties of the 8S complex. By the use of specific immunoadsorbents to AFP and by competitive studies with several tritiated estrogens as well as with pure AFP, the transition of the antigenic and the binding properties of the 8S complex toward those of serum AFP has been demonstrated after dissociation of the complex in 0·4 M KCl solutions.

G. **Aspects of steroid receptor biochemistry applicable to clinical problems,** ETIENNE-EMILE BAULIEU, Lab Hormones, 94270 Bicêtre, France

Intracellular, high affinity ($K_{D\,eq.}$ approx. 0·1 nM) specific binding proteins found in steroid hormone target cells are called steroid receptors.

(1) *Complexity of receptor.* Besides the already known cytosoluble Rc and nuclear KCl-extractable Rn_S receptors, there are nuclear binding sites RN, insolubilizable by any buffer and indifferent to exposure to DNAase and RNAase. From the Rn_i containing pellet, mild trypsin treatment can release a binding unit similar to the 4S fragment obtained by the same enzymatic treatment from soluble receptor. Hormone dependent, temperature and salt accelerated, "acidophilic activation" of Rc may explain the physiological $Rc \rightarrow Rn_S$ transformation and transfer. The significance of Rn_i in terms of interaction with DNA and gene expression, and within the receptor cycle in target cells, will be discussed with reference to the rat uterus and the chick liver and oviduct systems. A phenomenological distinction between "nuclear acceptor" and "executive" sites of the steroid receptors will be proposed.

(2) *Plurality of receptors per cell.* In 2 mouse cell lines, MI_1 (from an androgen dependent mammary tumor, the growth of which is inhibited by estrogens) and L-929 (fibroblasts, the growth of which is altered by corticosteroids), two sex steroid receptors are present, and androgen receptor RA and an estrogen receptor RE. RE, besides estradiol, binds non-steroidal synthetic estrogen diethylstilbestrol (DES) but not androgens, while RA, besides testosterone and androstanolone, binds