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 pre-treatment 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

estradiol somewhat but very little DES. Evidence has been obtained that the 2 binding sites do not belong to the same macromolecule. Results are interpreted as giving a molecular basis for interpreting antihormonal effects and for studying interactions of receptors with the genetic apparatus.

(3) *Regulation of receptors.* A classical phenomenon, the priming of estrogen action by estradiol in uterus, may be explained by the estrogen induction of the progesterone receptor synthesis. Moreover, estradiol induces the synthesis of its own receptor, and progesterone inhibits this induction, while progesterone increases the synthesis of the estrogen receptor in estrogen deprived endometrium. These observations are compatible with results reported under (2), since they suggest the presence of 2 receptors within uterine cells, for estradiol and progesterone, respectively.

The effects of steroid hormones on the intracellular distribution and the apparent half life of their own receptors have also been studied. In particular, progesterone seems to accelerate an apparent "inactivation" of its receptor.

The physiological changes observed in the uterus of the guinea-pig during the estrus cycle, and of the rat during early pregnancy, have been compared to the mechanisms studied in model conditions.

H. Oestrogen and androgen receptors in human breast cancer, H. MAASS, Department of Obstetrics and Gynaecology, University of Hamburg, Hamburg 20, Germany

Oestrogen and androgen receptors are routinely determined in specimens from primary and metastatic breast cancers. Agar gel electrophoresis (Wagner) has been used for determining the cytoplasmic oestrogen and 5α-DHT receptors. Specimens are called "positive" if the difference at the anodic peak is more than 100 c.p.m.

By this definition significant amounts of oestrogen receptors were found in 50% of primary cancers in the premenopausal group and 62% in the postmenopausal group. The rates in metastatic tissue are 22% and 39% respectively. The rate of specimens with 5α-DHT receptors is lower, in our material ranging between 20% and 25%.

Quantitatively the oestradiol binding ranges from 12·0–340·0 fmol/mg tissue protein or 18·0–268·0 fmol/mg DNA. The reference to the DNA content may be helpful in specimens with low receptor content.

Regarding clinical correlations to oestrogen receptor determinations at present 124 treatment trials had been evaluated. The rate of objective remissions in the "positive" group is 43/61, in the "negative" group 3/63. The number of patients who are evaluable regarding correlations to DHT receptors are low: remissions in DHT "positive" 4/5, in DHT "negative" 6/20.

In premenopausal patients there is a low concentration of available cytoplasmic receptor sites following the 12th day of the menstrual cycle.

Experiments on DMBA tumors showed that treatment of the animals with high doses of oestradiol is followed by a heavy decrease of oestrogen receptor contents. There is some evidence that the mechanism for the replenishment of new receptor protein is disturbed.

Similar observations had been made after treatment of the animals with ergocornin.

29. Appearance of nuclear estradiol receptor in perfused chicken liver, U. JOSS, Friedrich Miescher-Institut, P.O. Box 273, CH-4002 Basel, Switzerland

Estradiol causes the appearance of a nuclear estradiol receptor (R_N) in the liver of immature chickens. A significant increase of R_N is observed less than 1 min after administration of 2 μg estradiol/kg chicken into the portal vein. Maximal levels of 80 ± 10 fmol/mg protein are obtained 10 min after the estradiol pulse. The appearance is not sensitive to inhibition of protein synthesis by cycloheximide. The fact that no cytoplasmic receptor has been found in chicken liver prompted us to look for an extracellular receptor or precursor for R_N which would be transported into the liver nucleus and transformed to R_N by the effect of estradiol. In order to test this hypothesis, livers of immature chickens were isolated and perfused with synthetic perfusates. We found that the time course of appearance and maximal levels of R_N were similar to those found in the intact animal even when the synthetic perfusate contained only BSA and purified bovine erythrocytes. From this the existence of an extracellular precursor for R_N can be excluded, unless it is a contaminant of the bovine erythrocytes. The precursor for R_N therefore appears to be intracellularly located.

31. Specific progesterone receptors in DMBA-induced mammary tumors, J. ASSELIN, F. LABRIE, P. A. KELLY and J. P. RAYNAUD, Medical Research Council Group in Molecular Endocrinology, Centre Hospitalier de l'Université Laval, Québec, G1V 4G2, Canada and Centre de Recherches Roussel-UCLAF, Romainville 93230, France

The growing evidence for a correlation between the hormonal dependence of neoplastic tissues and the presence of specific hormone receptors led us to investigate the possible presence of specific progesterone receptors in dimethyl benzanthracene-induced mammary tumors in the rat. After homogenization in 3 vol. (w/v) of 25 mM Tris–HCl (pH 7·4), 1·5 mM EDTA, 10 mM thioglycerol and 10% glycerol (buffer A), the 105,000 *g* supernatant was used for binding studies with the highly potent synthetic progestin [^{3}H] R-5020 (17,21-dimethyl-19-nor-4,9-pregnadiene-3,20-dione). As measured by the charcoal assay after incubation for 15 h at 0°C, a significant level of soluble progesterone receptors is found in approximately 90% of tumors from untreated tumor-bearing animals. The level of progesterone receptors is 80 ± 20 fmol of [^{3}H]-5020 bound/mg of cytosol protein. As evidenced by sucrose gradient analysis, specific binding of [^{3}H]-5020 migrates at 7–8S. Specificity of the progesterone receptor was studied by both sucrose gradient and charcoal adsorption. [^{3}H]-5020 binding is competed by unlabelled R-5020, progesterone and a variety of synthetic progestins in a way very similar to the competition of [^{3}H]-5020 binding to the uterine progesterone receptor in rat. At a 100-molar excess, estradiol-17β and 5α-dihydrotestosterone inhibit [^{3}H]-5020 binding to the 7–8S component by 30 and 20%, respectively, while cortisol and dexamethasone are without effect. The potential significance of the level of progesterone receptors is indicated by our recent findings of a marked decrease of the level of this receptor after ovariectomy, a treatment accompanied by important regression of the tumors, and by the stimulatory effect of progesterone treatment upon tumor development.

32. The determination of the oestradiol receptor in normal and in neoplastic human mammary tissue, S. FUMERO, A. MONDINO, G. ZANOLO, Istituto di Ricerche Biomediche Antoine Marxer, RBM, Ivrea, V. AIMONE, C. CAMPAGNOLI, M. PERONA, II Clinica Ostetrica and Ospedale S. Anna, Torino