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

The concentration of the oestradiol receptor was determined by the DCC method, which we modified and simplified. The mammary tissue, at a 4:1 ratio (v/w) with Tris–EDTA buffer, was homogenized for 30 sec at 4°C. The homogenate was centrifuged at 24,000 *g* for 45 min at 4°C. The supernatant was incubated with 50 nM [^{3}H]-oestradiol both in the presence and in the absence of DES to differentiate the specific sites only. According to this method, positive values are considered those greater than 0·3 pmol/g of tissue, 6 pmol/g of protein, and over 40% BI%; negative values are considered those below 0·1 pmol/g of tissue, 3 pmol/g of protein and under 25% BI%. Intermediate values are considered very low or borderline. Our study was performed on 90 mammary carcinomas, 17 metastatic carcinomas and lymph nodes, 18 benign tumours, and 17 healthy parts and lymph nodes. All of the 17 healthy parts and lymph nodes proved negative for the receptor. Of the 18 benign tumours, 13 were negative, 4 borderline, and one positive. Of the 17 metastases, 8 proved negative and 9 positive. With only one exception, the presence or the absence of the receptor in the primary carcinomas was also confirmed in the lymph nodes. Of the 90 primary carcinomas, 60 were positive, equivalent to 66·6%, while 7, equivalent to 7·7%, proved to have very low or borderline receptor. In premenapausal patients, the incidence of negative carcinomas is higher. The contrary is true in postmenapausal patients. The concentration of the receptor tends to increase with age. Borderline and negative cases manifest a greater degree of cellular differentiation if classified histologically according to the WHO (1972).

33. Characterization and regulation of the progesterone receptor in the mouse uterus during the estrous cycle and during gestation, D. PHILIBERT and J. P. RAYNAUD, Centre de Recherches Roussel-Uclaf, 93230 Romainville, France

The variations in progesterone binding sites in the mouse uterus have been measured during the estrous cycle and during gestation and compared with plasma hormone levels. Uterine binding sites were assayed by the Dextran-coated charcoal exchange technique using a progestin-specific tag, R 5020 (17,21-dimethyl-19-nor-4,9-pregnadiene-3,20-dione) since this compound does not bind to CBG and since the cytoplasmic R 5020-uterine–receptor complex dissociates slower than the progesterone–receptor complex. Sucrose density profiles of the R 5020-complex from ovariectomized estradiol-primed mice showed a 7–8S peak not only following incubation *in vitro*, but also following injection *in vivo*, whereas progesterone gave a very marked 4S peak and only a slight hump in the 7–8S region which decreased with time. The cellular concentration of progestin binding sites reached a maximum at proestrus (1·4 pmol/mg protein). Three weeks following ovariectomy a value of approximately 0·3–0·4 pmol/mg protein was recorded. A single injection of 3 μg estradiol or moxestrol to these ovariectomized mice induced a rapid increase in the number of sites, which 36 h later reached a maximum of 1·2 pmol/mg protein, equivalent to the value recorded at proestrus, and then decreased with a half-life of 4 days. The simultaneous injection of 1 mg progesterone inhibited this increase by approximately 70%. In pregnant animals a minimum number of binding sites was recorded around mid-pregnancy; after this time, the number of sites increased until parturition. These studies suggest that the variations in progesterone receptor levels are mediated by events under the control of both estradiol and progesterone secretion.

34. Oestrogen-induced DNA synthesis in rat and human uteri, and in human uterine carcinoma, R. E. LEAKE, Department of Biochemistry, University of Glasgow, Glasgow G12 8QQ, Scotland

Oestrogen enters the nucleus of a target cell in conjunction with a specific protein receptor. This hormone–receptor complex then elicits various responses, both short- and long-term. To develop an understanding of the nature of the links between short- and long-term responses we have recently demonstrated that the continued presence of the hormone–receptor complex in the nucleus is essential for the induction of the 18 h peak of glucose transport (measured as the uptake of 2-deoxyglucose and its conversion to 2-deoxyglucose phosphate) but not for the 24 h stimulation of DNA synthesis (measured as the incorporation of [^{3}H]-Me thymidine into acid precipitable material, corrected for fluctuations in endogenous pool sizes). Experiments using cycloheximide suggest that the intermediate in the case of the stimulation of DNA synthesis is a labile protein, the properties of which are currently under investigation. These studies are currently being extended to human uterine carcinoma. Initially, we have measured receptor levels by using the Clark exchange assay on nuclear fractions, thereby eliminating any problems arising from non-translocateable cytoplasmic receptors. Suspensions of free cells have been made from explants of carcinomata and their ability to incorporate [^{3}H]-Me thymidine measured. Results are being coordinated with patient records.

35. High affinity steroid binding to bovine adrenal cortex cytosol, C. COCHET, P. M. MARTIN, P. H. ROLLAND and E. M. CHAMBAZ, Université Scientifique et Médicale, Grenoble, France

Soluble macromolecules have been suggested as intracellular carriers for steroid hormone precursors in the adrenal cortex; on the other hand, the possibility that this tissue is a target site for sex steroids has been put forward (M. O. Wilma *et al., Biochem. J.* **140** (1974) 495). In this work, the binding of a number of steroids to macromolecules of the bovine adrenal cortex cytosol was examined by the differential dissociation technique. Whereas low affinity binding with pregnenolone, progesterone (and their 17α-hydroxy analogs), deoxycorticosterone, deoxycortisol and aldosterone, a high affinity association was observed with dexamethasone (DXM), cortisol, testosterone and estradiol. The charcoal adsorption technique yielded an apparent association contant Ka of $\sim 10^8 M^{-1}$ for estradiol and testosterone, with a binding capacity in the range of 10^{-13} mol/mg proteins for the high affinity system. Study of the binding specificity showed that DHT was a better ligand than testosterone and that estradiol as well as ethinyl estradiol were effective competitors.

Further characterization of the corticosteroid binding moiety showed a high affinity for DXM (Ka $\sim 10^8 M^{-1}$); cortisol and corticosterone could displace the bound DXM whereas testosterone and estradiol were inactive. A blood contamination could be ruled out since no high affinity DXM binding could be detected in bovine plasma. The DXM binder appeared as a 7–8S moiety upon density gradient centrifugation in low salt buffer and chromatographed on Sephadex G-200 as a macromolecule of about 150,000 daltons M.W. The biological significance of such a glucocorticoid binder in the adrenal cortex will need further studies to be understood.