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.