than that at pregnancy. Very low levels (<1·0 fmol/mg protein) of ER in tumors from late lactating animals were associated with tumors that regressed during early lactation but were not reactivated. In contrast, 8 of the 10 tumors from the reactivated tumor group contained various levels of ER (>1·5 fmol/mg protein). Furthermore, decrease of ER was accompanied by regression of tumors after ovariectomy, ovariectomy-adrenalectomy, or anti-estrogens (nafoxidine hydrochloride). Concomitant increase of ER and growth rate of tumor was observed in animals treated with prolactin or low levels of estrogen. Serial biopsies of the same tumor at different stages of hormonal therapy or throughout pregnancy and lactation confirmed that the changes in ER levels were related to tumor growth patterns. The changes in ER levels of tumors during lactation differ from that of normal breast and uterine tissues. These results substantiate the hypothesis that ER is hormonally regulated as was demonstrated previously, and that ER levels may be of paramount importance to the growth and arrest of hormonally dependent cancer of the breast. Finally, that the sensitivity to high and low levels of hormones or their combinations, and that the mechanism of action of these hormones may likely be different in neoplasm and normal tissues. (Supported by NIH 5 MOI RR-00334 and the Cammack Trust Fund).

20. The effects of testosterone and estradiol-17β on DNA synthesis in human breast cancer and in rat DMBA-induced adenocarcinoma, H. HORN, A. GEIER, I. S. LEVIJ and M. FINKELSTEIN, Department of Endocrinology, Hebrew University Hadassah Medical School, Jerusalem, Israel

The effects of testosterone (20 μg/ml) and of estradiol-17β (1 μg/ml) on DNA synthesis were examined in maligant and non-malignant human breast grown in organ culture. Whereas in 14 out of 15 cases of benign breast tissue, the steroids inhibited the incorporation of [^{3}H]-thymidine into DNA, the effect on the malignant tissue was variable. Thus, testosterone (4/17 cases) or estradiol-17β (9/17 cases) stimulated the incorporation of [^{3}H]-thymidine into DNA in the cancerous tissue. The response of the uninvolved tissue of the cancer patients also differed from the response of the benign tissue. In organ culture of DMBA-induced adenocarcinoma in the rat, testosterone (20 μg/ml) inhibited the DNA synthesis in 7 out of 14 tumors. Estradiol-17β (1 μg/ml) inhibited the synthesis in 2 tumors but had no effect in the remaining 10. *In vivo*, 7 tumors out of 10 regressed following castration. In 4 out of 5 tumors which showed regression after castration, growth was stimulated by injecting the rats with estradiol-17β (5 μg/d during 3 weeks). Thus, whereas a pharmacological dose of estradiol-17β had by large no effect on the tumor *in vitro*, it had a stimulatory effect on its growth *in vivo* in rats in which tumor growth was inhibited following castration.

21. Progesterone and estradiol binding sites in human breast carcinoma, J. P. RAYNAUD, M. M. BOUTON and D. PHILIBERT, Centre de Recherches Roussel-Uclaf, 93230 Romainville, France, J. C. DELARUE, F. GUERINOT and C. BOHUON, Institut Gustave-Roussy, 92290 Villejuif, France

The possible hormone-dependence of 59 human mammary tumours was investigated by concomitantly measuring estradiol and progesterone binding sites on the assumption that progesterone receptor, normally induced by estradiol, may be taken as a criterion of estrogen responsiveness. Total, and not only free, binding sites were assayed by the Dextran-coated charcoal exchange technique (incubation 20 h at 0°C) using estradiol and R 5020 (17,21-dimethyl-19-nor-pregna-4,9-diene-3,20-dione) labelled with high specific activity. R 5020 is an extremely potent progestin not bound by CBG, but specifically and strongly bound by the cytoplasmic progestin receptor with which it forms a complex more stable than the progesterone–receptor complex. Estradiol and R 5020 bind to human mammary tumours with intrinsic dissociation constants of 0·09 ± 0·01 nM and 0·10 ± 0·06 nM respectively. Fifty-nine tumours were studied and in 14 instances results were compared to values recorded for normal mammary tissue from the same patient. This comparison revealed the difficulty of establishing a threshold level as a criterion of possible hormone responsiveness. On the basis of a threshold level of 100 fmol/g tissue, 14 tumours contained no sex steroid receptor, 11 contained estradiol receptor only, 5 progesterone receptor only and 29 both receptors. The full significance of these determinations will only become clear when the responsiveness of these patients to endocrine therapy is known. Moreover, only when other hormone receptors, such as the androgen and glucocorticoid receptors, have been screened in malignant mammary tissue and only when it has been established that the general mechanisms of hormone action (nuclear translocation of the cytoplasmic complex, nuclear response . . .) in normal and malignant tissue are identical, will it be feasible to select suitable clinical treatment on the basis of standardized biochemical assays with any degree of certainty.

22. The competitive action of 16β-ethyl estradiol on the binding of estrogen receptor in human breast cancer, H. TAKIKAWA and M. KURIHARA, Institute of Endocrinology, Gunma University, Maebashi, Japan

The presence of estrogen receptor in human breast cancer has been demonstrated by a number of investigators. It is accepted that some anti-estrogens inhibited the binding of estradiol-17β with estrogen receptor. Data will be presented on the competitive action of 16β-ethyl estradiol on the binding.

Breast cancer tissues were obtained from female patients after menopause, immersed in liquid nitrogen and excised after removal of the surrounding fat and connective tissue. The frozen tissue was crushed and pulverized. The tissue powder was mixed with 0·01 M Tris–HCl buffer, stirred in the cold and then centrifuged at 105,000 *g*. The supernatant was charged into a CNBr-activated Sepharose column coupled with anti-human rabbit serum and eluted with the buffer. The protein fraction except blood serum component was obtained. For further purification of the protein fraction a column electrophoresis in polyacrylamide gel and an electrofocusing in Ampholine column were employed. The effluent solution which corresponded to a major peak was dialysed and concentrated with Diaflo membrane filtra-

Table to Abstract No. 23

Receptor	<55 yr (N=10)	>55 yr (N=11)	(Wilcoxon-test)
Glucocorticoid	0–165 (60)	0– 300 (83)	no significant difference
Estrogen	43–515 (58)	42–7360 (870)	$P<0·01$
Androgen	0–155 (73)	0– 810 (183)	$P<0·05$

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