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)	$P<0·05$