of DNA synthesis and cell proliferation and that the function of nuclear receptor is concerned only with the regulation of the autophagic process. The latter view receives inferential support from observations on the growth behavior of human mammary and prostatic cancers. For example, whereas one tumour may display hormonal dependence, manifested as tumour regression induced by endocrine ablative therapy, another tumour may display hormonal responsiveness, manifested as tumour regression induced by endocrine additive therapy. However, since neither dependence nor responsiveness of mammary tumours is expressed in the absence of receptor proteins for steroid-hormones, it is likely that in both cases tumour regression hinges on the function of a common receptor-dependent mechanism.

With the present level of understanding, the control of proliferative growth by steroid-hormone is best visualized in terms of a model consisting of three regulatory elements assumed to be components of the cellular genome. It is proposed that, first, an initiator gene is responsible for switching on DNA synthesis and cell proliferation in the presence of an adequate concentration of steroid-hormone; second, a nullifier gene is responsible for switching off DNA synthesis and cell proliferation when the organ reaches a normal size, and accounts for negative feedback; and third, an autophage gene programs a cell for its own eventual destruction by capacitating the autophagic mechanism, perhaps through the formation of an inactive steroid–receptor complex. A fall in the concentration of hormone below levels required for the maintenance of a differentiated cell stimulates autolysis and removal of cells; it is conceivable that this effect depends on the function of a receptor molecule while is transformed from an inactive to an active state by declining hormonal concentration.

Whether endocrine therapy will result in carcinostatic or carcinocidal effects can probably be predicted by determining both the concentration of steroid-hormone and of receptor protein within the nucleus of the tumour cell. However, even on this basis some autonomous tumours will be indistinguishable from dependent tumours, and to improve the response rate to therapy, other methods which do not rely on either steroid-hormone or receptor measurements are needed to identify such resistant tumours.

A large number of autonomous tumours do not contain cytoplasmic receptor proteins and fail to transport steroid-hormones across the nuclear membrane. In respect to this group of neoplasms, a matter of practical importance that requires clarification is whether there is any potential for controlling cell proliferation with methods that would promote the entry of steroid-hormones into the nucleus of the autonomous cell to activate homeostatic processes such as those which suppress DNA synthesis or stimulate cellular autolysis. Clinical experience derived from the treatment of human mammary and prostatic cancers suggests that in selected cases the application of high doses of steroid may be sufficient for this purpose, but compounds which increase the permeability of the nuclear membrane to the passage of steroid-hormones should be sought.

In summary, growth of a normal hormone responsive organ appears to be ordered by the function of three constraint mechanisms which are sensitive to the intranuclear concentration of steroid-hormone. For the complete expression of these constraint mechanisms several properties underlying hormonal responsiveness must be manifested by the cell, including the presence of cytoplasmic receptor, the ability to transfer steroid-hormone into the nucleus, the competence to form nuclear receptor, and the fidelity of the interaction between steroid-hormone and chromatin.

Cytoplasmic receptor is not an exclusive indication of hormonal dependence or hormonal responsiveness *in vivo*, but its presence is associated with enhanced ability of the cell to incorporate steroid-hormone into the nucleus. Steroid-hormone is required for the initiation of DNA synthesis and cell proliferation, and nuclear receptor may not be required for these responses. On the other hand, it is possible that the function of the latter molecule is concerned with negative feedback or cellular autolysis.

18. Hormone-responsive mammary tumours in GR-mice
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Continuous treatment of castrated female GR-mice with estrone and progesterone leads to the appearance of mammary tumours within 3–4 months. Such tumours usually are hormone dependent, i.e. they are only transplantable in castrated mice if these animals are given estrone and progesterone. During serial transplantations in hormone-treated animals the tumours progressively lose their responsiveness towards estone and progesterone, and finally they become autonomous. We have obtained evidence from estrogen receptor assays that the hormone-responsive mammary tumours are mixed populations of hormone-dependent cells (which contain estrogen receptor) and autonomous cells (which are practically devoid of estrogen receptor). The hormone-dependent tumour cells do not multiply in the absence of estrone and progesterone, but the autonomous cells multiply in the absence of these hormones. The finding that the mammary tumours lose their hormone responsiveness after repeated serial transplantations appears to be due to the faster multiplication of the autonomous cells in the tumour as compared to the hormone-dependent cells. We have extended these studies to characterize these two types of mammary tumour cells in more detail. The investigations include comparative morphological studies of hormone-responsive and autonomous tumours, assays of various steroid receptors, assays of virions and antigens of the mammary tumour virus, and assays of peroxidase in the tumour cells. A pilot study has been started to determine whether the GR-mouse system can be used as a model to investigate optimal conditions for combined hormonal and chemotherapy.

19. Growth pattern and estrogen receptor levels of dimethylbenzanthracene-induced tumor during pregnancy and lactation,
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Hormonal influences on tumor growth and estrogen receptor (ER) in breast cancer of rats induced by dimethylbenzanthracene were studied during pregnancy and lactation to elucidate the mechanism of prolactin-estrogen action and interaction. During pregnancy, palpable tumors were stimulated to grow rapidly and new active sites were initiated. Just prior to or immediately after delivery, rapid regression of tumor was observed. Some regressed tumors were reactivated, some continued to regress, and some remained static during the latter part of lactation. When cytoplasmic ER of tumors from pregnant rats was examined by the Dextran-charcoal assay, only one of the 23 identified adenocarcinomas did not contain measurable levels of ER. Significant reduction of ER occurred just prior to delivery and during early lactation. Among the 30 regressed tumors from early lactating rats, 19 had very low ER levels ($<1{\cdot}5$ fmol/mg protein) while the rest had significant but lower ER levels

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-

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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)	$P<0·05$