Eur J Cancer Clin Oncol, Vol. 19, No. 6, pp. 835–842, 1983.
Printed in Great Britain.

0277-5379/83$3.00+0.00

Close Correlation between Progesterone Receptor Concentration and Hormonal Sensitivity in DMBA-induced Mammary Tumours of the Rat*

B. G. MOBBS

Department of Surgery, Medical Sciences Building, University of Toronto, Toronto, Canada M5S 1A8

Abstract—*Cyclic administration of oestrogen alone and with progesterone to ovariectomized rats bearing DMBA-induced tumours resulted in more uniform cytosol oestrogen and progesterone receptor (ER and PgR) concentrations in the uteri and a stronger correlation between ER and PgR concentrations in the tumours than in a group of naturally cycling animals. Using this model, the concentration of PgR was a better indicator of tumour response to ovariectomy and hormonal restimulation than the concentration of ER. In ten unequivocally responsive adenocarcinomas receptor values, expressed as* fmol/mg *cytosol protein, were: ER* $47 \pm S.D.$ *19, PgR* 246 ± 134, *PgR:ER* 5.9 ± 3.0, *compared with ER* 18 ± 2, *PgR* 33 ± 8 *and PgR:ER* 1.8 ± 0.3 *in three autonomous adenocarcinomas. All 3 parameters were significantly different between the two groups* ($0.02 < P < 0.05$), *but there was overlap in the ER values, whereas no overlap occurred in the PgR values or the PgR:ER ratios. Ten other adenocarcinomas showed a different sensitivity to restimulation than to ovariectomy and six tumours were fibroadenomas or of mixed histopathology. For the whole tumour population the response to hormonal restimulation at the time of excision suggested that a PgR concentration* >60 fm/mg *protein* combined with *an ER concentration* >20 fm/mg *protein is necessary for the maintenance of hormonal sensitivity. Using these criteria, response was related to receptor concentrations in 93% of the tumours.*

INTRODUCTION

EXPERIMENTAL mammary cancer induced in the rat with 7,12-dimethylbenz(a)anthracene (DMBA) has in many ways proven to be a useful model for the study of human breast carcinoma. In particular, these tumours show a varying degree of responsiveness to ovarian hormone deprivation, and several studies have shown that oestrogen receptor (ER) concentration in the tumour cytosols correlates moderately well with tumour regression after ovariectomy [1–4]. However, in the same studies occasional unresponsive tumours have appeared which have high ER concentrations: these may correspond to the ER-positive unresponsive tumours observed in women. Horwitz *et al.* [5] suggested that in these tumours ER is nonfunctional and that progesterone receptor (PgR) may be a suitable marker for the functional activity of ER since induction of PgR synthesis is known to be dependent on oestrogen [6].

In designing an experiment to investigate the relationship between ER, PgR and response to ovariectomy of DMBA-induced tumours it is necessary to produce a uniform hormonal environment in the experimental animals. In untreated animals circulating endogenous hormone levels undergo fluctuations during the oestrous cycle, resulting in cyclic changes in concentration and cellular distribution of receptor protein [7], as well as changes in the proportion of binding sites occupied by endogenous steroids. Any variation in assayable receptor content inherent in the tumours would thus be modulated by differences in their hormonal environment, resulting in difficulties in the interpretation of

Accepted 6 December 1982.
*This work was supported by grant No. 341 from the Ontario Cancer Treatment and Research Foundation.

Abbreviations: DMBA, 7,12-dimethylbenz(a)anthracene; DCC, dextran-coated charcoal: ER, estrogen receptor; PgR, progesterone receptor; R5020, promegestone: 17,21-dimethyl-19-nor-4,9-pregnadiene-3,20-dione.

data. Elimination of endogenous ovarian hormones by ovariectomy may in the short term increase assayable cytosol receptor sites by emptying occupied sites and/or releasing nuclear sites into the cytosol, but if hormonal deprivation continues for more than a few days (i.e. for long enough to determine tumour response to ovariectomy), cytosol receptor concentrations become depleted [2, 8, 9]. Biopsy of the tumours at a known phase of the oestrous cycle followed by ovariectomy and monitoring of regression of the remaining part of the tumour is not wholly satisfactory. Firstly, it is difficult to establish with certainty the phase of the oestrous cycle without taking daily vaginal smears throughout several cycles, and secondly, tumour biopsy may cause oedema and/or bleeding at the tumour site, making accurate measurement of tumour size difficult. In this laboratory a third approach for establishing a uniform hormonal environment has been adopted, i.e. ovariectomy followed by restimulation with oestrogen and progesterone after an interval of several days during which tumour regression can be monitored. This approach has the additional advantage of allowing a second evaluation of hormonal sensitivity during restimulation. In an earlier publication [3] injection of an oestradiol/progesterone combination three times weekly was used for restimulation. However, as progesterone is known to reduce the concentration of both ER and PgR [10, 11], in this study a cyclic regimen of oestradiol alone followed by combined oestradiol and progesterone has been used in an attempt to maximise concentrations of both receptors. The receptor concentrations in the tumours and in the uteri of the treated animals has been compared with those in a group of animals undergoing natural oestrous cycles. The results show that interanimal variation in uterine receptor concentration, particularly in ER concentration, is greatly reduced in the treated rats and that correlation between ER and PgR concentrations in the tumours of the treated animals is closer than in the cycling animals. The data also support the clinical evidence that PgR together with ER values may give a better indication of hormonal responsiveness than ER concentration alone [12].

MATERIALS AND METHODS

Reagents

Oestradiol-[2,4,6,7-^{3}H] (specific activity 90–115 Ci/mmol), promegestone-[17α-methyl-^{3}H] (R5020; specific activity 70–80 Ci/mmol) and radioinert R5020 were obtained from New England Nuclear Corporation, Boston, MA. The labelled steroids were diluted to 10 μCi/ml in redistilled benzene: ethanol (9:1) on arrival and aliquots of this stock solution were evaporated under nitrogen on the day of receptor assay. Estradiol, progesterone, cortisol, 5α-dihydrotestosterone and 7,12-DMBA were obtained from Sigma Chemical Co., St. Louis, MO; dextran T70 from Pharmacia, Montreal; charcoal (Norit A) from Matheson, Coleman and Bell, Norwood, OH; and Bray's scintillator and PCS were obtained from New England Nuclear Corp. and Amersham Corp., Arlington Heights, IL respectively.

Tumour induction and treatment

Mammary tumours were induced in female Holtzman rats (Holtzman, WI) by the gastric instillation of 15 mg DMBA in 1.5 ml sesame oil at age 50–52 days followed by a second dose of 10 mg 1 week later. Tumours (often multiple) began to appear 4–5 weeks after dosing and then growth was monitored by weekly measurement of the largest diameter and that at right angles to it with calipers. When the largest tumour in each animal reached 1.5–2.0 cm in mean diameter the animals were treated in one of two ways: (i) (21 rats) animals were killed by decapitation under light ether anaesthesia. The uterus and all tumours with mean diameter $>$0.9 cm were removed. Cystic and necrotic areas were cut away and representative portions of all tumours were fixed in buffered formalin for histological examination. The remaining tumour tissue and the uterus were frozen and stored in liquid nitrogen for not more than 1 month before receptor assays were carried out. Fourteen animals in this group developed 33 tumours of a suitable size for assay; (ii) (52 rats) animals were ovariectomized and tumour size was monitored for a further 2 weeks to establish response to ovariectomy. Restimulation was then begun with a weekly cyclic regimen of 2 μg oestradiol on day 1 followed by 2 μg oestradiol and 8 mg progesterone on days 3 and 5. The next cycle started on day 8. These injections were given subcutaneously in 0.4 ml sesame oil. Injections were continued for 2–4 cycles to establish response to restimulation. A final subcutaneous injection of 2 μg oestradiol in saline was given two days before death in order to stimulate PgR synthesis while allowing clearance of oestradiol from the animal so that cytosol ER would be available for the free site assay. Animals with tumours which did not regress after ovariectomy were given the hormone injections for two cycles and also the final oestradiol injection so as to establish the same hormonal status in all animals at the time of death. Seventeen animals in this group developed 29 tumours large enough for assay. Tumour and

uterine tissue from the treated animals was processed as described for the cycling animals. Tumours in the latter group of animals were used 7–20 weeks after DMBA administration; in the treated animals tumours were used over a somewhat wider time-span after dosing (11–35 weeks) due to the time necessary for establishing response.

Receptor assays

A single saturating dose incubation followed by DCC separation of free and bound steroid was used for both receptor assays. All procedures subsequent to tissue pulverization were carried out at 0–4°C.

Preparation of cytosol

Tumours were pulverized using a Thermovac tissue pulverizer (Thermovac Industries, Copiague, NY) cooled with liquid nitrogen. The resulting powder was homogenized in TE buffer (10 mM Tris, 1.5 mM EDTA, brought to pH 8.0 with 0.1 N HCl) using approximately 1.0 ml buffer for every 100 mg tissue powder. Homogenization was carried out in an ice-cooled vessel using 4 × 10-sec bursts with a Polytron homogenizer at medium speed, with 50-sec cooling intervals.

The resulting homogenate was centrifuged for 10 min at 6500 ***g*** to provide a crude supernatant which was then recentrifuged for 60 min at 100,000 ***g*** in a Beckman L5-50 ultracentrifuge to give the pure cytosol fraction. Twenty-five microlitres of the latter were removed for protein determination by the method of Lowry *et al.* [13] and the pellet from the first low-speed spin was used for tissue DNA determination by the diphenylamine method [14].

Oestrogen receptor assay

Replicate 0.2-ml aliquots of cytosol were diluted 1:1 with TE buffer containing [^{3}H]-oestradiol with or without radioinert oestradiol. The final incubation concentration was 1 nM [^{3}H]-oestradiol ± 400 nM oestradiol, to permit correction for low affinity binding. Incubation was carried out overnight at 0–4°C. Separation of free from bound steroid was carried out by incubation for 15 min at 4°C with 0.5 ml DCC (0.05% charcoal and 0.05% dextran in TE buffer) followed by centrifugation at 2500 ***g*** for 10 min. The supernatant was then recentrifuged. Supernatant (0.5 ml) was added to 10 ml PCS:toluene (2:1 v/v) and counted in a Beckman LS-7500 scintillation counter for 20 min or to 2% error.

Progesterone receptor assay

Replicate 0.2-ml aliquots of cytosol were diluted 1:1 with TEGS-1 buffer (10 mM Tris, 0.5 mM EDTA, 40% glycerol, 0.5 M sucrose, brought to pH 8.0 with 0.1 N HCl) containing radioinert cortisol and 5α-DHT with or without radioinert R5020. The final incubation concentrations were 5 nM [^{3}H]-R5020, 500 nM cortisol and 50 nM 5α-DHT ± 500 nM R5020. Incubation was carried out for 2 hr at 4°C. Since no glycerol was present during homogenization and cytosol preparation it is likely that PgR was dissociated from endogenous ligand and that this assay quantitates total cytosol PgR [15]. Separation of free from bound steroid was carried out as in the oestrogen receptor assay except that DCC was suspended in TEGS-2 buffer (10 mM Tris, 1 mM EDTA, 20% glycerol and 0.25 M sucrose, pH 8.0). After centrifugation 0.5 ml of the supernatant was counted in 10 ml Bray's scintillator for 20 min or to 2% error.

The results of both assays were expressed as fmol (10^{-15} mol) per mg DNA and fmol per mg cytosol protein.

RESULTS

In the cycling animals 18 of the 33 tumours were increasing in size at the time of autopsy, 13 had maintained a constant size for at least 2 weeks and 2 appeared to be spontaneously regressing. However, all had a similar histopathology and appeared to be active adenocarcinomas with large, granular nuclei. Mitotic figures were observed in all but one tumour. Oestrogen and progesterone receptor values for the tumours and uteri from these animals are plotted in Fig. 1. These are expressed as $\log_{10}$ fmol/mg DNA to permit direct comparison between uterine and tumour tissue, which differ widely in their cellularity. A significant correlation was observed between these values in both tissues, but the range of values for both receptors was higher in the uteri. There appeared to be no relationship between the receptor values and the rate of tumour growth at the time of autopsy.

In tumours from the treated animals more varied histopathology was observed. Twenty-three tumours were adenocarcinomas, 2 were fibroadenomas and 4 were of mixed histopathology, having both adenocarcinoma and fibroadenoma components. Hormonal sensitivity was also varied. Seventeen of the adenocarcinomas regressed after ovariectomy, but only ten of these regrew during restimulation. The others remained small, although histologically two contained active-looking areas. Three adenocarcinomas appeared to be autonomous and did not respond to ovariectomy. Three adenocarcinomas became palpable only during restimulation. One of the fibroadenomas regressed

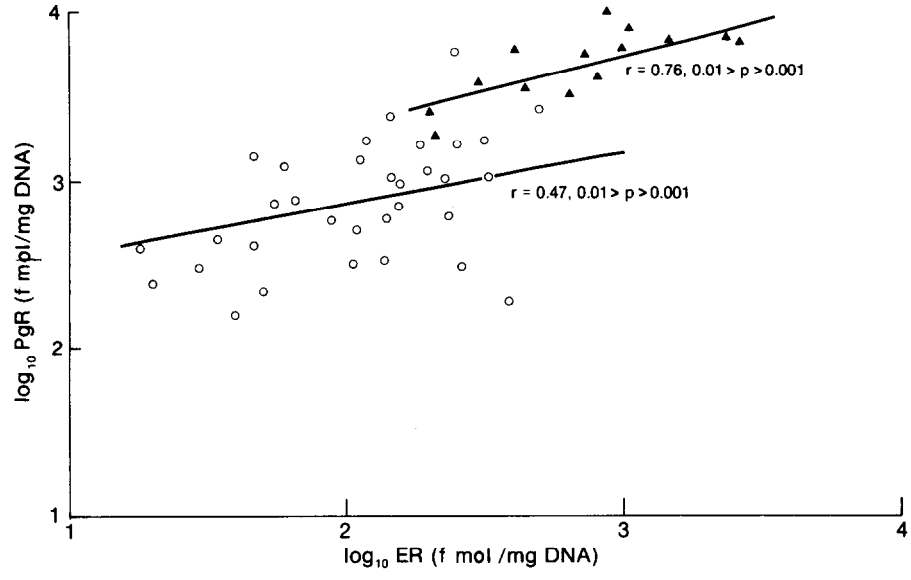

Fig. 1. Oestrogen and progesterone receptor concentrations in uteri and DMBA-induced mammary tumours in rats undergoing natural oestrous cycles. ▲ Uteri; O tumours.

after ovariectomy and did not regrow on restimulation: the other continued to grow throughout both hormonal manipulations. The 4 tumours of mixed histopathology also showed a variety of growth patterns: three regressed after ovariectomy, but only one of these regrew during restimulation. The remaining tumour continued to grow after ovariectomy and during restimulation. In spite of the variations in histopathology and growth pattern, the significance of the correlation between ER and PgR values in the tumour population as a whole was higher than that in the cycling animals, and the interanimal variation in the receptor values of the uteri was greatly reduced: this applied particularly to the ER values (Fig. 2). The oestrogen receptor concentration in uteri from the cycling animals was 937 ± S.D. 749 fmol/mg DNA vs 1352 ± 353 fmol/mg DNA in the treated animals. Progesterone receptor values were 5356 ± 2221 fmol/mg DNA in the cycling animals vs 3018 ± 1011 fmol/mg DNA in the treated animals.

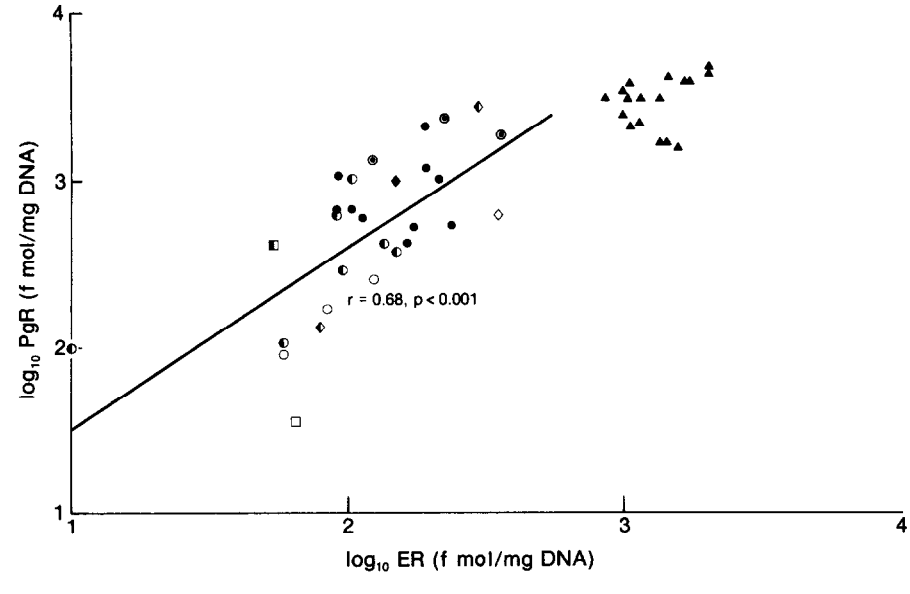

Fig. 2. Oestrogen and receptor concentrations in uteri and DMBA-induced mammary tumours in ovariectomized rats after cyclic treatment with oestrogen alone and with progesterone. Solid symbols, tumours which responded to both ovariectomy and hormonal restimulation; open symbols, tumours which did not respond to ovariectomy; closed/open symbols, tumours which responded to ovariectomy but did not regrow on restimulation. ●○◐ Adenocarcinomas; ◧◨ fibroadenomas; ◆◇◈ tumours with mixed histopathology; ⊙ adenocarcinomas which became palpable on restimulation; ▲ uteri.

The cytosol protein and tissue DNA yields from the tumours in the treated animals were 49.8 ± 11.8 mg/g tissue and 12.4 ± 2.6 mg/g tissue respectively. Although the correlation between ER and PgR concentrations in the tumour population as a whole was highly significant, subpopulations were observed in which receptor concentrations were related to growth pattern. Receptor values and PgR:ER ratios are shown in Table 1. Some of the highest values were observed in the three adenocarcinomas which became palpable only during restimulation. When ER and PgR values and the PgR:ER ratios of the 10 adenocarcinomas which showed unequivocal hormonal sensitivity were compared with those which were autonomous throughout both ablative and additive hormonal manipulation it was observed that although there was an overlap in the range of ER concentrations in the two groups, no overlap occurred in PgR concentration or in PgR:ER ratio. The means for these values in responsive and autonomous tumours were significantly different. Expressed as fmol/mg DNA, PgR concentrations were 878 ± 494 for responsive vs 168 ± 81 for autonomous tumours ($P = 0.035$). ER concentrations were not significantly different at the 0.05 level (160 ± 55 for responsive tumours vs 90 ± 36 for unresponsive tumours). Expression of the results as fmol/mg cytosol protein increased the significance of the mean values: for the unequivocally responsive vs the unequivocally autonomous tumours the values were as follows: for PgR, 246 ± 134 vs 33 ± 8 ($P = 0.021$), and for ER, 47 ± 19 vs 18 ± S.D.

Table 1. Receptor concentrations and tumour response to hormonal manipulation in treated animals

Tumour type	ER (fmol/mg DNA)	PgR (fmol/mg DNA)	ER (fmol/mg protein)	PgR (fmol/mg protein)	PgR:ER	Response to ovariectomy/restimulation
Adenocarcinomas						
Regressed after	97	1055	23	251	10.9	+/+
ovariectomy, regrew on	198	2081	55	578	10.5	+/+
restimulation	93	674	20	144	7.2	+/+
	102	657	24	154	6.4	+/+
	191	1141	53	319	6.0	+/+
	115	603	53	277	5.2	+/+
	219	1075	54	263	4.9	+/+
	177	543	50	154	3.1	+/+
	161	399	53	133	2.5	+/+
	246	554	82	186	2.3	+/+
Mean ± S.D.	160 ± 55[e]	878 ± 494[b]	47 ± 19[c]	246 ± 134[d]	5.9 ± 3.0[a]	
Did not regress						
after ovariectomy	83	165	19	37	2.0	-/0
	129	251	20	38	1.9	-/0
	59	89	16	24	1.5	-/0
Mean ± S.D.	90 ± 36[e]	168 ± 81[b]	18 ± 2[c]	33 ± 8[d]	1.8 ± 0.3[a]	
Regressed after	105	1000	23	218	9.5	+/-
ovariectomy but did	10	91	4	37	9.1	+/-
not regrow on	92	638	19	131	6.9	+/-
restimulation	97	287	20	59	3.0	+/-
	151	360	23	56	2.4	+/-
	59	103	19	33	1.7	+/-
	131	171	24	32	1.3	+/-
Mean ± S.D.	92 ± 47	306 ± 322	19 ± 7	81 ± 70	4.8 ± 3.6	
Not palpable	124	1378	32	355	11.1	0/+
before restimulation	288	2299	38	382	10.1	0/+
	358	1864	123	639	5.2	0/+
Mean ± S.D.	237 ± 117	1847 ± 461	64 ± 51	459 ± 157	8.8 ± 3.2	
Mixed histopathology	154	1075	57	396	7.0	+/+
	300	2791	54	499	9.3	+/-
	79	136	16	28	1.8	+/-
	356	644	22	39	1.8	-/-
Fibroadenoma	54	421	17	132	7.8	+/-
	66	35	6	3	0.5	-/-

[a] $P = 0.044$; [b] $P = 0.035$; [c] $P = 0.030$; [d] $P = 0.021$; [e] $P > 0.05$.

2 ($P = 0.030$). Thus in these tumours expression of the results as fmol/mg protein appeared to give better discrimination between responsive and unresponsive tumours than expression as fmol/mg DNA. PgR:ER ratios were 5.9 ± 3.0 for responsive vs 1.8 ± 0.3 for autonomous tumours ($P = 0.044$).

In addition to the unresponsive adenocarcinomas, one wholly unresponsive fibroadenoma and one autonomous tumour of mixed histology had PgR concentrations <50 fmol/mg protein and PgR:ER ratios <1.9. Thus all five tumours which had not responded to ovariectomy had low PgR concentrations and PgR:ER ratios <2.0, although the ER concentrations in two were in the 'responsive' range (≥20 fmol/mg protein).

Four of the six adenocarcinomas which underwent regression after ovariectomy but did not regrow during restimulation had PgR concentrations <50 fmol/mg protein and/or ER:PgR ratios <2.5 at the time of death; at this time these tumours appeared to have lost their hormonal sensitivity but were not growing autonomously. Two other adenocarcinomas which showed the same lack of growth on restimulation had concentrations of both receptors and PgR:ER ratios in or very close to the range of those in unequivocally responsive adenocarcinomas. Lack of hormonal sensitivity in these two could not have been due to loss of receptor activity.

All PgR concentrations >60 fmol/mg protein occurred in tumours with ER concentrations ≥19 fmol/mg protein: thus none of these tumours fell into the ER–PgR+ category that has been observed in human breast carcinomas.

DISCUSSION

This study has shown that it is possible to produce a uniform hormonal environment in rats bearing DMBA-induced tumours while maintaining tumour growth and concentration of assayable oestrogen and progesterone receptors comparable with those in animals undergoing natural oestrous cycles. The majority of the tumours in rats so treated were adenocarcinomas, but a few fibroadenomas and tumours with both adenocarcinoma and fibroadenoma components were observed. After ovariectomy and during restimulation a variety of growth patterns were observed: these did not appear to be related to the tumour type. In spite of these variations the relationship between the oestrogen and progesterone receptor concentrations (expressed as $\log_{10}$ fmol/mg DNA) in the group of tumours as a whole was highly significant ($P < 0.001$). When the tumours were grouped according to their response to hormonal manipulation it was observed that twenty-one tumours had ER concentrations ≥20 fmol/mg protein at the time of tumour excision. However, only 14 (67%) of these were hormonally sensitive at this time. The other seven would be classified as ER-positive non-responders: five of these had PgR concentrations <60 fmol/mg protein. Thus, using a 'cut-off' point for ER of 20 fmol/mg cytosol protein *combined with* a 'cut-off' point for PgR of at least 60 fmol/mg protein, the correlation of responsiveness at the time of tumour excision with receptor content reached 93% (27/29 tumours). It was of interest that of the 10 tumours (7 adenocarcinomas, 1 fibroadenoma and 2 tumours with mixed histopathology) which regressed after ovariectomy but did not respond to restimulation 9 contained ER concentrations above or very close to 20 fmol/mg protein at the time of excision. In five of these PgR concentrations were <60 fmol/mg protein, suggesting that loss of sensitivity due to loss of functional activity of ER had occurred between ovariectomy and restimulation. Four tumours had borderline-to-high ER values combined with high concentrations of PgR, so that loss of sensitivity was more likely to be due to loss of post-receptor function. Only one of the tumours with loss of sensitivity had low values for both receptors. Thus loss of hormonal sensitivity (as measured by changes in tumour size) occurring during hormonal deprivation apparently may be due to loss of ER, loss of ER function (as assessed by PgR concentration) or to events other than alterations in cytosol receptor content. However, in these tumours loss of sensitivity did not result in autonomous growth, at least within the time of observation.

It is of interest that some of the highest receptor concentrations and PgR:ER ratios were observed in tumours which were not palpable before ovariectomy but appeared to arise during stimulation by exogenous hormones. Possibly endogenous hormone levels were not high enough to promote tumour growth in these animals.

In this investigation the best correlation of hormonal sensitivity with receptor content is given by concentrations of ≥20 fmol/mg cytosol protein for ER combined with 60–130 fmol/mg protein for PgR. As no PgR concentrations in this series of tumours fell between these values it is not possible to be more precise. It is of interest to compare these 'cut-off' points with those used in this laboratory for human breast carcinomas. For premenopausal patients the values used for ER and PgR are 10 fmol/mg cytosol protein and 45–60 fmol/mg cytosol protein respectively, i.e. approximately half the 'cut-off' concentration of the DMBA-induced tumours. Based on values

from 100 breast carcinomas (unselected except for the exclusion of tumours with lymphocytic infiltration), the mean cytosol protein and DNA yields were 31.8 ± 8.8 mg/g tissue and 4.8 ± 2.5 mg/g tissue respectively. The protein:DNA yield ratio was 7.7 ± 3.2. The protein:DNA yield ratio was 4.2 ± 1.2 in the DMBA tumours, reflecting the greater cellularity of these tumours compared to most human breast tumours and the relatively smaller amount of cytosol protein contributed by non-epithelial elements. Taking this into account, it becomes necessary to halve the receptor concentrations expressed per mg protein in the DMBA tumours in order to obtain 'cut-off' points which would be comparable to those in human breast carcinomas. If this is done, 'cut-off' points of 10 fmol/mg cytosol protein for ER and 30–65 fmol/mg protein for PgR are obtained, which are in fact equivalent to those used in this laboratory for human specimens. The higher 'cut-off' point for PgR may be due to the fact that, as mentioned in Materials and Methods, our assay is likely to be measuring total PgR rather than unoccupied receptor only, as is the case with ER.

This investigation shows that in the DMBA rat mammary tumour model ER and PgR values used together accurately reflect the hormonal sensitivity of the tumours in animals in which fluctuations in circulating hormonal levels have been eliminated by standardised treatment. Under these conditions the PgR:ER ratio may indicate the degree of functional activity of the ER present. In addition to providing a method for the establishment of a uniform hormonal environment resulting in receptor concentrations similar to those occurring naturally, this investigation gives experimental support to the clinical data which suggest that measurement of PgR concentration results in greater accuracy of prediction of the hormonal sensitivity of breast cancer than oestrogen receptor assay alone.

Acknowledgement—Skilled technical assistance was provided by Mrs S. Parnell.

REFERENCES

1. BOYLAN ES, WITTLIFF JL. Specific estrogen binding in rat mammary tumors induced by 7,12-dimethylbenz(a)anthracene. *Cancer Res* 1974, **35**, 506–511.
2. KOENDERS AJM, GEURTS-MOESPOT AG, ZOLINGEN SJ, BENRAAD THJ. Progesterone and estradiol receptors in DMBA-induced mammary tumors before and after ovariectomy and after subsequent estradiol administration. In: MCGUIRE WL, RAYNAUD JP, BAULIEU EE, eds. *Progesterone Receptors in Normal and Neoplastic Tissues*. New York, Raven Press, 1977, 71.
3. MOBBS BG, JOHNSON IE. Estrogen-binding *in vitro* by DMBA-induced rat mammary tumors: its relationship to hormone responsiveness. *Eur J Cancer* 1974, **10**, 757–763.
4. NOMURA Y, ABE Y, INOKUCHI K. Specific estrogen receptor and its relation to response to oophorectomy in rat mammary cancer induced by 7,12-dimethylbenz[a] anthracene. *Gann* 1974, **65**, 523–528.
5. HORWITZ KB, MCGUIRE WL, PEARSON OH, SEGALOFF A. Predicting response to endocrine therapy in human breast cancers: a hypothesis. *Science* 1975, **189**, 726–727.
6. TOFT DO, O'MALLEY BW. Target tissue receptors for progesterone: the influence of estrogen treatment. *Endocrinology* 1972, **90**, 1041–1045.
7. SHIH A, LEE C. Fluctuations in levels of cytosol and nuclear receptors in rat mammary tumors during the estrous cycle. *Endocrinology* 1978, **102**, 420–425.
8. MOBBS BG. Uptake of [^{3}H] oestradiol by dimethylbenzanthracene-induced rat mammary tumours regressing spontaneously or after ovariectomy. *J Endocrinol* 1969, **44**, 463–464.
9. GIBSON SL, HILF R. Influence of hormonal alteration of host on estrogen-binding capacity in 7,12-dimethylbenz(a)anthracene-induced mammary tumors. *Cancer Res* 1976, **36**, 3736–3741.
10. CLARK JH, HSUEH AJW, PECK GJ. Regulation of estrogen receptor replenishment by progesterone. *Ann NY Acad Sci* 1977, **286**, 161–176.
11. VU HAI MT, LOGEAT F, WAREMBOURG M, MILGROM E. Hormonal control of progesterone receptors. *Ann NY Acad Sci* 1977, **286**, 199–207.
12. EDWARDS DP, CHAMNESS GC, MCGUIRE WL. Estrogen and progesterone receptor proteins in breast cancer. *Biochim Biophys Acta* 1979, **560**, 457–486.
13. LOWRY OH, ROSEBROUGH NJ, FARR AL, RANDALL RJ. Protein measurement with the Folin phenol reagent. *J Biol Chem* 1951, **193**, 265–275.

14. DISCHE A. Colour reactions of nucleic acid components. In: CHARGAFF E, DAVIDSON JN, eds. *The Nucleic Acids.* New York, Academic Press, 1955, Vol. I, 287.
15. PICHON MF, MILGROM E. Characterization and assay of progesterone receptor in human mammary carcinoma. *Cancer Res* 1977, **37**, 464–471.