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Letter to the Editor

Progestogen and Oestrogen Receptor Activity in Ovary-Dependent and Ovary-Independent Tumours of the Rat

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ALTHOUGH mammary tumours containing oestrogen receptors are more likely to respond to endocrine therapy, the presence of the oestrogen receptor does not guarantee hormonal sensitivity [1, 2]: a critical, minimal concentration of receptors must be present [3–5]. Jensen has used the phrase "oestrogen receptor-rich" to distinguish such tumours from those lacking that concentration, "receptor-poor". An alternative approach has been suggested by McGuire and his colleagues [6], who noted that the synthesis of the progestogen receptor is oestrogen-dependent and thus the presence of progestogen receptor activity is a marker of a functional oestrogen receptor.

We have now examined three types of rat mammary tumour for both oestrogen and progestogen receptor activities: these are two lines of transplantable mammary tumour, TG-3 and TG-5, which are now ovary-independent [7] and dimethylbenz[*a*]anthracene (DMBA)-induced mammary tumours which are predominantly ovary dependent.

Tumours were excised for use when 1.0–2.5 cm in diameter. Cytosol for receptor analysis was prepared as previously described]9], except that glycerol (10% v/v) and monothioglycerol (1% v/v) were included in the homogenisation buffer. Oestrogen receptor activity was determined using 100 μl portions of tumour cytosol as previously described [8]. Progestogen receptors were determined by incubating overnight, on ice, either (a) 50–200 μl cytosol with 0.22 nM [7α-^{3}H]Org-2058 (sp. act. 18.3 Ci/mmol) ± 0.22 – 11.1 nM non-radioactive Org-2058 in Tris–glycerol buffer, or (b) 200 μl diluted cytosol (1.5 × with buffer) with 0.5 nM [6, 7-^{3}H] R5020 (sp. act. 56.5 Ci/mmol) ± 1–1000 nM non-radioactive R-5020 in Tris buffer. Free and bound hormone were separated by adsorption on charcoal [(a) 500 μl 0.15% w/v or (b) 400 μl 0.625% w/v. The data were analysed according to Scatchard [9].

Sucrose density gradient analysis of the progestogen receptor was carried out as described for the oestrogen receptor [10] with minor modifications: glycerol (10% v/v] was included in the homogenisation buffer and gradients, and the [^{3}H]progestogen and the non-radioactive progestogen concentrations were, respectively, 8 and 32.2 nM for Org-2058 and 1.4 and 23.6 nM for R-5020.

A total of 31 tumours (17 DMBA-induced, 7 TG-3 transplanted and 7 TG-5 transplanted) were examined for progestogen receptor activity using [^{3}H] Org 2058 and for oestrogen receptor activity: in 15 tumours (5 of each type) progestogen receptor activity was also determined using [^{3}H] R-5020. The results are listed in Table 1. Oestrogen receptors were detected in all three types of tumour, the mean level detected being significantly higher in the DMBA-induced tumours than in either line of transplantable tumour ($P < 0.01$ by Wilcoxon Rank Sum test). The average dissociation constant of binding was 0.22×10^{-10} ($N = 31$, range $0.06–0.68 \times 10^{-10}$) molar. Progestogen receptor activity was detected in one of seven TG-5 transplantable tumours, in four of seven TG-3 transplantable tumours and in all seventeen DMBA-induced tumours when assays were carried out with

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Table 1. *Oestrogen and progestogen receptor activities in three types of rat mammary tumour*

Tumour type	Oestrogen receptor activity*		Progestogen receptor activity*			
			Using [^{3}H]Org-2058		Using [^{3}H]R5020	
	Fraction R+	fmoles/mg tumour	Fraction R+	fmoles/mg tumour	Fraction R+	fmoles/mg tumour
	Sensitivity	0.05†	—	0.15‡	—	0.25§
TG-5 transplants	7/7	0.41 (0.11–0.66)	1/7	0.18 (0–0.18)	1/5	1.7 (0–1.7)
TG-3 transplants	7/7	0.46 (0.31–0.65)	4/7	0.36 (0–0.40)	4/5	2.1 (0–2.1)
DMBA-induced	17/17	3.24 (0.77–7.08)	17/17	26.9 (0.15–144.9)	5/5	40.6 (7–86)

*Fraction R+ = No. tissues with detectable receptor activity/total tissues studied.
fmoles/mg tumour = mean (and range) of concentrations found in R+ tissues.
Sensitivities derived arbitrarily as follows:
† > 50 cpm displaced by addition of excess, nonradioactive steroid.
‡ > 150 cpm displaced by addition of excess, nonradioactive steroid.
§ > 200 cpm displaced by addition of excess, nonradioactive steroid.

[^{3}H]Org-2058. There was generally good qualitative agreement between the results of the two assays for progestogen receptors using different ligands, and with [^{3}H] R-5020, levels were detectable in one of five TG-5 transplanted tumours, four of five TG-3 transplanted tumours and all of five DMBA-induced tumours. The levels of activity were very low in all the transplanted tumours and in one (using Org-2058) or two (with R-5020) of the DMBA-induced tumours. Dissociation constant of binding was 1.7×10^{-10} molar (mean $n = 19$, range $0.25–6.3 \times 10^{-10}$) with the assay employing Org-2058, and 8.6×10^{-10} molar with that employing R-5020. Quantitatively, progestogen receptor levels were considerably higher in the DMBA-induced tumours than in the transplantable tumour lines ($P < 0.01$ by Wilcoxon Rank test).

The progestogen receptor extracted from DMBA-induced tumours was found to have a sedimentation constant of approximately 8–9s using either radioligand, but no peak in binding of [^{3}H]progestogen could be demonstrated in the transplantable tumours. Amongst various compounds examined, only progestogen competed with [^{3}H]Org-2058 for binding to cytosol from a DMBA-induced tumour; the binding specificity of [^{3}H] R-5020 is already well established [11, 12]. In our hands, these two progestogens were effectively equipotent (Relative Binding Affinities: R5020 96%, Org-2058 100% using [^{3}H]Org-2058, and R5020 100%, Org-2058 100% using [^{3}H]R5020). Neither progestogen bound detectably to cytosol, prepared from the prostate of a male rat and containing androgen receptors.

In our previous studies of oestrogen receptor activity [7, 8, 10], oestrogen translocation [13], steroid metabolism [14, 15] and sensitivity to hormones in culture [16] in these tumour types, we have not found such a striking difference as that seen here: whilst oestrogen receptor levels were seven times higher in the predominantly ovary-dependent, DMBA-induced tumours than in the ovary-independent transplantable tumours, progestogen receptor levels were 19–75 times higher. We conclude that these differences, seen between carcinogen-induced tumours and the advanced generations of transplantable tumours, provide further support for the hypothesis of McGuire *et al.* [6] that progestogen receptor activity may represent a useful index of hormonal sensitivity in breast cancers.

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