

Cryostat section assay of oestrogen and progesterone receptors in meningiomas: a clinicopathological study

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SUMMARY Oestrogen receptors and progesterone receptors were measured in the cytosols from cryostat sections of 45 meningiomas from 40 patients (12 men, 28 women) using an isoelectric focusing technique. Near facsimile adjacent sections from the same tissue blocks were stained and examined to determine the histological subtype of the neoplasms. Appreciable levels of progesterone receptor (> 10 fmol/mg cytosol protein) were present in 24 (53.3%) of the neoplasms, but no clinically important oestrogen receptor was detected in any of the tumours. Competitive binding studies on control tissues confirmed the specificity of the assay procedures. No correlation was found between progesterone receptor state and the age, sex, or menopausal state of the patients, or the histological subtype and site of the neoplasms. Four of the patients studied had multiple intracranial neoplasms, which in two were of differing progesterone receptor state. The presence of specific progesterone receptor in meningioma cytosols raises the possibility of hormonal manipulation in the treatment of this group of neoplasms.

Meningiomas account for 13–18% of intracranial neoplasms and 25% of intraspinal neoplasms.¹ Two thirds of meningiomas occur in women,¹ and for many years it has been known that meningiomas in women may enlarge and become symptomatic during periods of hormonal flux,² particularly during pregnancy³ and in the luteal phase of the menstrual cycle.⁴ An increased incidence of meningiomas occurs in women with breast carcinoma,⁵ a neoplasm that often contains oestrogen receptors and progesterone receptors and which exhibits hormonally dependent growth.⁶ Oestrogen receptor and progesterone receptor have recently been found in normal leptomeninges,⁷ the tissue from which meningiomas are thought to arise in man.

These observations have prompted several investigations into the presence of oestrogen receptor and progesterone receptor in meningiomas,^{8–26} and it has been postulated that the presence of such receptors might be of prognostic and therapeutic value, as for breast carcinoma. All of the assays performed to date have measured oestrogen receptor and progesterone receptor in the cytosol fractions from tissue homogenates of meningiomas. A wide range of results has been obtained, and although an associ-

ation between steroid hormone receptor state and histological subtype of meningioma has been claimed,^{19,20} this observation has not been consistent.^{21,23} Histological typing of meningiomas is usually performed on an adjacent portion of tumour to the sample taken for homogenisation and steroid receptor assay. These two portions of tissue, however, may not be exact replicates as meningiomas are heterogeneous neoplasms which may exhibit considerable histological variability.¹

We overcame this problem by assaying oestrogen receptor and progesterone receptor in the diffusate of cryostat sections of meningiomas, thus allowing simultaneous histological analysis and steroid hormone receptor assay on adjacent near facsimile samples. This method is based on the observation that unoccupied oestrogen receptor and progesterone receptor readily diffuse from unfixed cryostat sections immersed in an aqueous medium without the need for mechanical homogenisation.^{27–29}

Material and methods

Forty five consecutive meningiomas were studied from 40 patients (28 women, 12 men) of mean age 55.5 years (age range 33–79). Four patients (three women, one man) had multiple tumours; of these, one

woman came from a family with neurofibromatosis with predominantly central affliction. The site of origin of each neoplasm was identified radiologically and confirmed at the time of surgery.

TISSUE COLLECTION AND STORAGE

Fresh tissue obtained during surgery was trimmed into blocks measuring about $10 \times 10 \times 5$ mm suitable for cryostat microtomy. These were snap frozen and stored at -70°C . Fresh uterus for positive control tissue was obtained from oophorectomised rats for oestrogen receptor assay and from rats killed one week after subcutaneous implantation of a pellet containing 12 mg hexoestrol (Boots Company Limited) for progesterone receptor assay. The control tissues were frozen and stored under identical conditions to the meningiomas.

CRYOSTAT SECTIONS AND RECEPTOR ASSAY

Cryostat sections for receptor assay were cut at $40 \mu\text{m}$, and two additional sections from each block for histology were then cut from the same tissue surface at $6 \mu\text{m}$ with minimal trimming. Both sections were stained by haematoxylin and eosin to permit classification of the histological subtype of each tumour. This was performed without knowledge of the receptor assay results.

Oestrogen receptor assay was performed on cytosols from the cryostat sections with incubation on ice for one hour with ^3H -oestradiol followed by trypsinisation as previously described in a method for breast carcinomas.³⁰ For progesterone receptor assay duplicate sets of cryostat sections ($40 \mu\text{m}$) to give a total section area greater than 1 cm^2 were collected on small glass coverslips and dropped into 70×9 mm plastic tubes containing $200 \mu\text{l}$ progesterone receptor buffer (5 mM KH_2PO_4 , 2 mM mercaptoethanol, 10% W/V glycerol, 0.05% W/V bovine serum albumin, 0.02% W/V NaN_3 , pH 7.4). The glass coverslips were then crushed gently, and a further $200 \mu\text{l}$ of progesterone receptor buffer containing $2 \times 10^{-9}\text{M}$ ^3H -ORG2058 (58 Ci/mM; Amersham International) was added immediately. The tubes were kept on ice for two hours during which they were gently agitated at 15–20 minute intervals. The tubes were then centrifuged at 2500 rpm for 10 minutes at 4°C . Supernatant enriched with cytosol ($300 \mu\text{l}$) was gently aspirated and added to a clean plastic tube containing $150 \mu\text{l}$ of dextran coated charcoal (1.0 g activated charcoal, 0.1 g dextran T-500, 100 ml progesterone receptor buffer). The mixture was agitated, incubated for five minutes on ice, and then centrifuged at 2500 rpm for 10 minutes at 4°C . Supernatant ($50 \mu\text{l}$) was aspirated, and the protein concentration measured by a modified Lowry method³¹ against a blank containing $50 \mu\text{l}$ progesterone receptor buffer alone. Two $\times 100 \mu\text{l}$ aliquots of the remaining supernatant were then subjected to isoelectric focusing.

DIFFUSION AND BINDING STUDIES

To estimate the rate of diffusion of receptor protein from cryostat sections progesterone receptor concentrations were measured in sections of control tissue (rat uterus) after immersion in buffer for two minutes, 15 minutes, 30 minutes, one hour, and two hours, before the addition of ^3H -ORG2058, as described above. To estimate the optimum incubation period for ligand-receptor binding after the addition of ^3H -ORG2058 cryostat sections of control tissue were incubated with the radioligand for five minutes, 15 minutes, 30 minutes, one hour, two hours, and three hours. The progesterone receptor concentration in the supernatant was then determined.

COMPETITIVE BINDING STUDIES

To investigate the specificity of binding of ORG2058 to cytosolic progesterone receptor incubations of section cytosols with $2 \times 10^{-9}\text{M}$ ^3H -ORG2058 were conducted either alone or in the presence of a 100 fold molar excess of either progesterone (Sigma), diethylstilboestrol (Sigma), hydrocortisone (Sigma), or testosterone (Sigma). Incubations were also performed with dexamethasone (Decadron; Merck, Sharp and Dohme) at concentrations of $2 \times 10^{-9}\text{M}$ and 10, 100 and 1000 fold molar excess. After incubation for two hours on ice the assay tubes were centrifuged, and the amount of receptor bound ^3H -ORG2058 measured.

ISOELECTRIC FOCUSING

Isoelectric focusing was performed on duplicate $100 \mu\text{l}$ aliquots of cytosol supernatant after incubation with ^3H -ORG2058 and dextran coated charcoal. Each sample was pipetted into a plastic application frame ($10 \times 6 \times 2$ mm) placed 10 mm from the cathodal electrode strip on the surface of a thin layer polyacrylamide gel ($245 \times 110 \times 1$ mm; 2.4% W/V ampholine, pH 3.5–9.5; LKB). The focusing conditions were similar to those used by Underwood *et al.*³⁰ After focusing for 90 minutes the gel along each sample track was cut into 5 mm slices, each of which was placed into a scintillation phial, to which was added 4 ml of HP/b scintillant (Beckman). The phials were then incubated in a water bath at 50°C for one hour with gentle agitation.

CALCULATION OF RECEPTOR CONCENTRATION

The radioactivity of each phial was measured by liquid scintillation, counting with quench monitoring by H number (external standard Compton Edge monitoring using ^{137}Cs). Counting was performed in an LS1801 automated liquid scintillation spectrometer (Beckman). A histogram of radioactivity against gel slice number was plotted for each sample and the cumulative height of the points in the single peak, typically located about 5 cm from the cathodal electrode strip (pH 6.2), was measured above the dia-

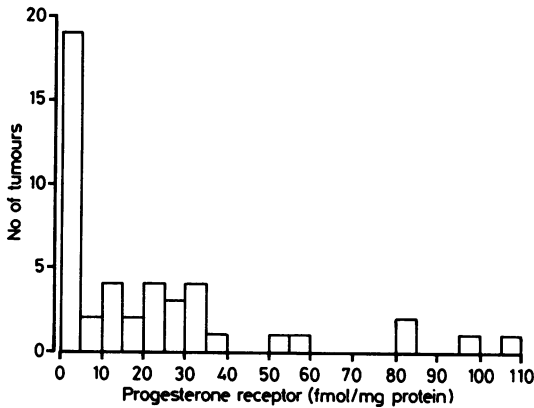


Fig. 1 Results of progesterone receptor assay. Nineteen neoplasms contained no detectable cytosolic progesterone receptor, while two neoplasms contained concentration of less than 10 fmol/mg cytosol protein.

grammatic baseline. The amount of ^3H -ORG2058 within the peak was calculated by computer, and the results were finally expressed in fmol/mg of cytosol protein. If the results for the duplicate assays from each tissue sample varied by more than 10% the assay was repeated.

SCATCHARD PLOT ANALYSIS

A measurement of the affinity of ^3H -ORG2058 for cytosolic progesterone receptor was performed. Cryostat section cytosol in progesterone receptor buffer (100 μl) was added to either 50 μl of unlabelled ORG2058 at a concentration of 150 nmol/l to esti-

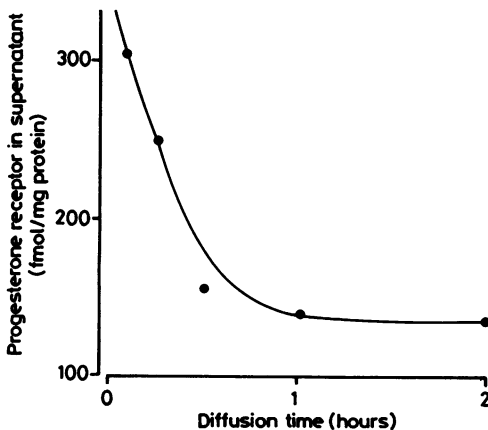


Fig. 2 Diffusion study shows rapid diffusion of progesterone receptor from cryostat section into aqueous buffer in the first few minutes after immersion. Progesterone receptor is labile and decays following prolonged immersion before ^3H -ORG2058 is added. After each interval a two hour incubation with radioligand was performed and cytosolic progesterone receptor measured by isoelectric focusing.

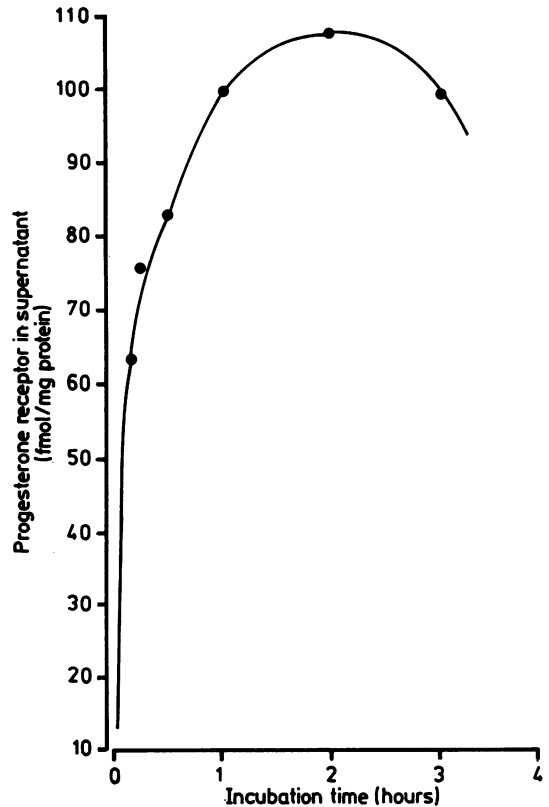


Fig. 3 Progesterone receptor binding study shows that optimal concentrations of progesterone receptor/radioligand complexes were detected by isoelectric focusing after a two hour incubation of cytosol with $2 \times 10^{-9} \text{ M}$ ^3H -ORG2058 following immersion of cryostat section in buffer for two minutes.

mate non-specific binding, or 50 μl of progesterone receptor buffer to measure the total binding of the radiolabelled ligand. The mixtures were incubated for 30 minutes on ice, then 100 μl of ^3H -ORG2058 at concentrations varying from 400–10000 fmol/ml was added. For each concentration of radioligand a blank specimen containing 100 μl of radioligand and 150 μl of progesterone receptor buffer, but no cytosol or unlabelled ORG2058, was prepared. Background counts were measured from specimens containing 250 μl of progesterone receptor buffer only. All of the above were incubated overnight at 4°C . Dextran coated charcoal (prepared as above) (200 μl) was then added followed by incubation on ice for 30 minutes. After centrifugation at 2000 rpm for 10 minutes at 4°C , 50 μl of the supernatant was removed for protein estimation. The concentration of bound radioligand in fmol/ml in the reaction was calculated and plotted against the bound/free ratio for each of the concentrations used.³²

Results

STEROID HORMONE RECEPTORS

Fig. 1 shows the results of progesterone receptor assays. Of the 45 neoplasms examined, 24 (53.3%) were positive for progesterone receptor (> 10 fmol/mg cytosol protein). Only one of the neoplasms contained detectable oestrogen receptor at a concentration of 2.3 fmol/mg cytosol protein; this result is not important—that is, (< 10 fmol/mg cytosol protein). Of the group of patients with multiple meningiomas, two women had neoplasms both positive and negative for progesterone receptor while one woman had two negative neoplasms for progesterone receptor and the man had two neoplasms positive for progesterone receptor.

DIFFUSION AND BINDING STUDIES

Fig. 2 shows the results of the diffusion study for progesterone receptor. Rapid diffusion of cytosolic receptor into the aqueous medium necessitates the addition of radiolabelled ligand for progesterone receptor assay as soon as possible after placing the cryostat sections into the buffer and crushing the glass coverslip. Fig. 3 shows the results of the binding study for ^3H -ORG2058 and progesterone receptor. An incubation period of two hours was deemed to give the most satisfactory results with this ligand and reflected similar experiences by other workers.³³

COMPETITIVE BINDING

Fig. 4 shows the results of the competitive binding experiment. No important level of competitive binding was found with any of the steroid hormones other than progesterone; in particular, no competitive binding with dexamethasone was found at any of the concentrations used.

SCATCHARD PLOT ANALYSIS

Fig. 5 shows the graph obtained from the results of a Scatchard plot analysis. The calculated K_D for the reaction is 0.704 nmol/l, indicating a high affinity reaction between the radioligand and the cytosolic progesterone receptor.

TISSUE STORAGE

No important difference between the results of receptor assays repeated on tissues stored for up to six months was found, indicating the stability of cytosolic progesterone receptor at the conditions of storage specified above.

HISTOLOGY

Table 1 shows the histological subtypes³⁴ of the neoplasms studied listed in relation to progesterone receptor state. Although the numbers are too small to permit statistical analysis, interestingly, the ana-

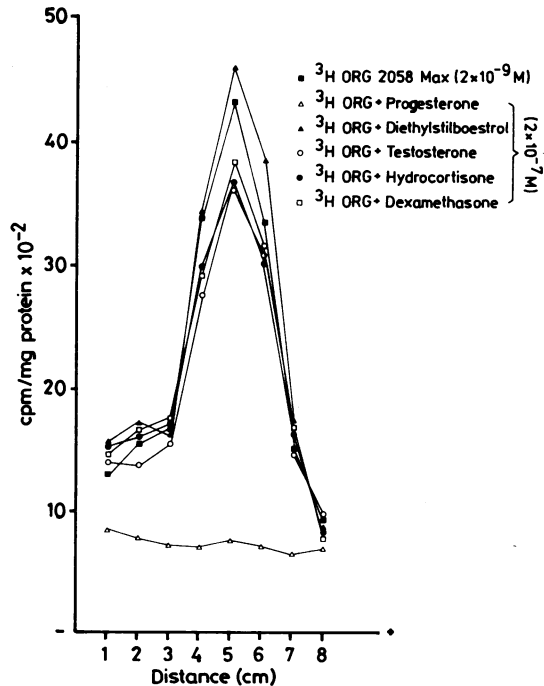


Fig. 4 Specificity of ^3H -ORG2058 for progesterone receptor is shown by competitive binding study where suppression of isoelectric focusing peak for progesterone receptor/radioligand complexes occurs only in presence of excess unlabelled progesterone. Incubations were performed with 2×10^{-9} M ^3H -ORG2058 alone (■) and in the presence of progesterone (Δ), diethylstilboestrol (\blacktriangle), testosterone (\circ), hydrocortisone (\bullet), and dexamethasone (\square), each in 100 fold molar excess (2×10^{-7} M).

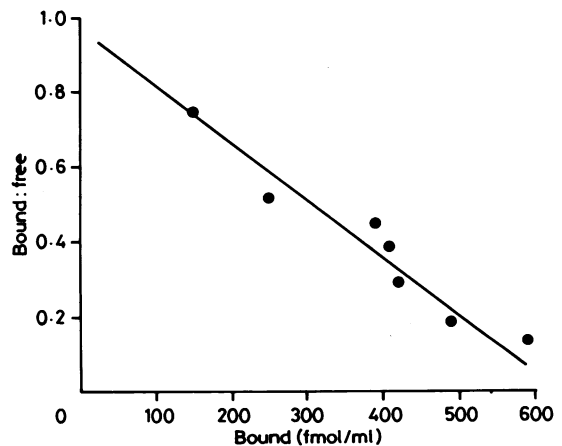


Fig. 5 Scatchard analysis of progesterone receptor/ ^3H -ORG2058 binding in meningioma cytosol shows a straight line plot of regression 0.922, giving a K_D of 0.704 nmol/l.

Table 1 *Histological subtype in relation to progesterone receptor state*

| Subtype (WHO) | Positive for progesterone receptor | Negative for progesterone receptor |
|---------------------|------------------------------------|------------------------------------|
| Transitional | 10 | 8 |
| Meningotheliomatous | 8 | 5 |
| Fibrous | 2 | 3 |
| Psammomatous | 3 | 0 |
| Angiomatous | 0 | 2 |
| Anaplastic | 0 | 2 |
| Haemangioblastic | 0 | 1 |
| Microcystic | 1 | 0 |

Table 2 *Site of neoplasm in relation to progesterone receptor state*

| Site | Positive for progesterone receptor | Negative for progesterone receptor |
|------------------|------------------------------------|------------------------------------|
| Convexity | 8 | 10 |
| Central basal | 6 | 1 |
| Parasagittal | 3 | 6 |
| Lateral basal | 4 | 2 |
| Intraventricular | 0 | 2 |
| Spinal | 3 | 0 |

plastic, angiomatous, and haemangioblastic neoplasms were all negative for progesterone receptor, whereas all the psammomatous neoplasms were positive for progesterone receptor. No significant differences in progesterone receptor state between the larger groups of transitional and meningotheliomatous neoplasms was found ($p > 0.05$, χ^2 test).

CLINICAL FEATURES

No significant difference was found between the age distributions of the groups of patients with neoplasms positive or negative for progesterone receptor ($p > 0.05$, χ^2 test). Among the women there was no correlation between menopausal state and the presence of progesterone receptors in the neoplasm ($p > 0.05$, χ^2 test). No significant difference was found between the sex distribution in the groups of patients with neoplasms positive or negative for progesterone receptor ($p > 0.05$, χ^2 test). Table 2 lists the sites of the neoplasms examined in relation to progesterone receptor state. No significant association was found between progesterone receptor state and neoplasms occurring in midline, lateral, or basal sites ($p > 0.05$, χ^2 test).

Discussion

The technique for steroid hormone receptor assay described in this study has an important advantage over previous methods entailing tissue homogenisation in that it facilitates accurate histological classification of an adjacent near facsimile section to the assayed sample. This is of particular importance

in meningiomas, which are often heterogenous in their histological appearances. In an attempt to overcome this problem when dealing with tissue homogenates some authors have classified meningiomas into typical or atypical histological subtypes.²⁵ The criteria used in the recognition of these groups, however, leave a considerable overlap between the more clearly defined histological subtypes and do not differentiate between locally infiltrative benign or anaplastic neoplasms. Other authors have claimed that tissue homogenates of presumed meningoendotheliomatous meningiomas contain more progesterone receptor than transitional or fibroblastic tumours,¹⁹ but no evidence to support this claim was found in the present study. From our results it seems that there is no relation between the histological subtype of the neoplasm and the steroid hormone receptor state, although it will be necessary to examine more cases of the rarer subtypes to fully assess this relation.

The presence of steroid hormone receptors in meningiomas was first investigated in 1979 by Donnell *et al* who reported the presence of oestrogen receptor in four out of six meningiomas.⁸ Since then many studies have been published on the presence of oestrogen receptor and progesterone receptor in tissue homogenates from meningiomas.⁹⁻²⁶ There is considerable disparity between the published results, some authors claiming to have found an appreciable number of neoplasms that were positive for oestrogen receptor,^{8-10 12 14 16 17 20 23 26} while others have found that most meningiomas were negative for oestrogen receptor.^{11 13 15 18 19 21 22 25} There does appear to be general agreement that progesterone receptor is present in meningiomas, although the incidence of positive neoplasms varies considerably from series to series. A recent review by Cahill *et al* found that the results of analysis of steroid hormone receptor in meningiomas fell into two main groups, depending on the presence of clinically important oestrogen receptor.²¹ Interestingly, most of the reports describing high concentrations of oestrogen receptor binding in a large proportion of neoplasms originate from France.^{9 12 20 23} Various theories have been put forward to explain this.^{21 23} These include differences in the techniques of tissue biopsy, freezing, duration of storage, and methods of homogenisation. These variables, however, have differed in other reports, which are unanimous in finding low or undetectable concentrations of oestrogen receptor. Another explanation for this discrepancy is the effect of preoperative treatment with dexamethasone on oestrogen receptor and progesterone receptor in meningiomas.¹² Poisson *et al* reported high concentrations of oestrogen receptor in tumours from patients who had been treated preoperatively with glucocorticoids.²³ These findings contrast with those from an earlier series from the same laboratory¹² and with a series from Sweden,¹⁴

same laboratory¹² and with a series from Sweden,¹⁴ in which pretreatment with dexamethasone apparently resulted in considerably lower concentrations of progesterone receptor. All the patients in the present report had received preoperative or intraoperative dexamethasone, and so we were unable to perform a similar comparison. The results of the competitive binding experiment, however, indicate that dexamethasone does not interfere with the progesterone receptor or oestrogen receptor assays used in our experiments.

It has also been suggested that differences in the radiolabelled ligands used in the various reported series might account for the differing results.²¹ Most of the assays to detect oestrogen receptor have been performed using ³H-oestradiol, although R2858 (11-beta-methoxy-17-beta-ethynylestradiol) was used as the oestrogen analogue by workers in France.^{9,12,20} It has been suggested that this ligand might offer a more accurate oestrogen receptor assay as it does not bind to human sex hormone binding globulin in the serum, and that binding to such serum proteins may lead to artefactually high results if ³H-oestradiol is used.³⁵ Surprisingly, the incidence of oestrogen receptor in meningiomas when measured using R2858 is much higher than when ³H-oestradiol has been used. All the previous reports used the synthetic analogue R5020 (³H-promegesterone) as the progesterone analogue in the progesterone receptor assay.⁸⁻²⁶ The analogue used in this study ³H-ORG2058 (16 alpha-ethyl-21-hydroxy-19-nor-[6,7-³H] preg-4-en-3,20 dione) was chosen because of its specific high affinity binding to progesterone receptor, resulting in a stable complex^{36,37} with low cross reactivity with other cytosol receptor sites and with serum corticosteroid binding globulin.³⁷ ³H-ORG2058 exhibits lower non-specific binding than R5020, which may bind weakly to cytosolic glucocorticoid receptors.³⁸ The results of the progesterone receptor assay in this report were broadly similar to those of several previous reports^{11,18,19,25} although direct comparisons are difficult because of differences in assay technique, measurement of receptors, and interpretation of a significant result.

Schwartz *et al* in a study of 26 meningiomas confirmed the presence both of oestrogen receptor and progesterone receptor, but because of an apparent lack of steroid binding specificity concluded that the receptor proteins found in meningiomas were unlikely to be specific steroid receptors.²⁴ The results of this and numerous other studies, however, seem to indicate otherwise. It is unlikely that the progesterone receptor activity in our study represents non-specific binding of radioligand to low affinity molecules. Isoelectric focusing is a technique that is eminently suitable for separating receptor bound radioligands from those that are non-specifically bound, especially when

working with small volumes as in the technique used in this study.³⁹ The isoelectric focusing profile of progesterone receptor in meningiomas was identical to that obtained in control tissues, and similar results have been obtained from human breast carcinomas.³³ Furthermore, the competitive inhibition studies showed the highly specific nature of the cytosolic progesterone receptor in meningiomas. Similar results were obtained by other authors,^{15,20} and Scatchard plot analysis both in this and other reports has further established the high affinity nature of cytosolic progesterone receptor in meningiomas.^{15,19,20}

An analysis of the clinical features of patients whose tumours were studied in this series confirms previous reports that there is no correlation between the presence of progesterone receptor and the age, sex, or menopausal state in the patients studied, or the site of the neoplasm. In the patients with multiple neoplasms it was interesting to note that no generalised predictions could be made regarding the steroid hormone receptor state of the tumours removed. One of the female patients in this series had a familial history of neurofibromatosis with predominantly central manifestations, details of which are published elsewhere.⁴¹ The presence of progesterone receptor in patients with multiple, recurrent, or extensively infiltrative neoplasms is of particular interest as it raises the possibility of hormonal manipulation as a form of treatment in adjunct to surgery. This possibility merits investigations *in vitro* before clinical trials are started.

The presence of cytosolic progesterone receptor in meningiomas in the apparent absence of oestrogen receptor has led to speculation that, in contrast to other cells, the synthesis of progesterone receptor in meningiomas may not be modulated by oestrogens acting through oestrogen receptor.²⁵ Alternatively, it has been suggested that meningiomas containing clinically important quantities of cytosolic progesterone receptor may also contain nuclear oestrogen receptor occupied by endogenous oestrogens which cannot be readily detected by current assay techniques.⁴² The presence of nuclear oestrogen and progesterone receptor in meningiomas has been reported,²⁶ but the relation of such receptors to free cytosolic receptors remains unclear. Further studies on the presence of nuclear receptors in meningiomas are required before accepting the presence of an oestrogen independent progesterone receptor in these neoplasms. The use of other techniques—for example, cellular localisation of oestrogen receptor using monoclonal antibodies—⁴³ may be of considerable value.

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