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Relationships Between Polyamine, Deoxyribonucleic Acid and Oestrogen Receptor Binding Site Concentrations in Human Breast Tumours

J.C. NORMAN, J.R. PUDDEFOOT,* ELIZABETH ANDERSON* and HANNELORE BRAUNSBERG

Department of Chemical Pathology, St Mary's Hospital Medical School, London W2 1PG *and* **Department of Biochemistry, Medical College of St Bartholomew's Hospital, London* EC1M 6BQ, *U.K.*

Abstract—*Concentrations of polyamines, DNA and oestrogen receptor (ER) binding sites have been estimated in 23 malignant and 2 benign breast tumours. Polyamine and DNA concentrations, expressed per unit wet weight of tissue, were significantly higher in ER-poor (<10 fmol/mg cytosol protein) than in ER-rich tumours. Spermidine/spermine ratios were similar in ER-poor and ER-rich tumours. There was a relationship between polyamine and DNA levels and when polyamine concentrations were expressed per unit weight of DNA those in ER-poor tumours did not differ from those in ER-rich tumours. Possible reasons for these findings are discussed.*

INTRODUCTION

ALTHOUGH their mode of action remains to be elucidated, polyamines appear to be involved in cell proliferation [1, 2]. Evidence has been presented that inhibition of ornithine decarboxylase, the enzyme which initiates the biosynthesis of these substances, can reduce the chemical induction [3–6] or growth [7] of rodent breast tumours and the oestrogen-stimulated growth of human breast cancer cells in culture [8]. Exposure to oestradiol of cultured cells increased ODC activity [9] and polyamine concentrations [10] and the antioestrogen tamoxifen decreased ODC activity as well as proliferation [9].

A recent report has presented evidence that the polyamine concentrations in human breast tumour tissues may be related to histological type and grade, time to recurrence of the disease and oestrogen receptor (ER) binding site concentration, and it was concluded that high levels of polyamines were associated with tumour aggressiveness [11]. We have analysed breast tumour tissues from 25 patients for polyamines, oestrogen receptor binding sites and deoxyribonucleic acid (DNA) and have found a close relationship between concentrations of these components.

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MATERIALS AND METHODS

Tissues

Samples of tumours were taken from 25 patients; 23 were found to be malignant and 2 benign. Tissues were frozen immediately in liquid nitrogen and stored in liquid nitrogen until assay.

Chemicals

Putrescine dihydrochloride (PUT.HCl), spermidine trihydrochloride (SPD.HCl), spermine tetrahydrochloride (SPE.HCl), 1,6-diaminohexane (DAH), 1,8-diaminooctane (DAO), trichloracetic acid (ACS reagent), calf thymus deoxyribonucleic acid (highly polymerized, sodium salt), activated charcoal (hydrochloric acid washed and untreated powder), high purity 3,5-diaminobenzoic acid (DABA), Tris(hydroxymethyl)aminomethane (Tris), monothioglycerol and diethylstilboestrol (DES) were from Sigma Chemical Co. (Poole, Dorset, U.K.); analytical grade sodium dihydrogenphosphate, ethylene diaminotetra-acetic acid (EDTA) and glycerol were from BDH Ltd (Poole, Dorset, U.K.); dextran T70 was from Pharmacia A.B. (Uppsala, Sweden); [2,4,6,7-^{3}H]oestradiol (sp. act. 85–110 Ci/mmol) was from Amersham International plc (Amersham, Bucks.); diethyl ether, water (HPLC grade), methanol (HPLC grade) and all inorganic chemicals were from Fisons (Loughborough, Leicestershire, U.K.); benzoyl chloride (gold label) was from Aldrich Chemical Co. (Gillingham, Dorset, U.K.); ethanol (ana-

lytical reagent) was from James Burrough (Witham, Essex, U.K.).

Free DAH and DAO were treated with hydrochloric acid to convert them to dihydrochlorides (DAH.HCl and DAO.HCl) and recrystallized from ethanol; stock solutions (10 mM in HPLC grade water) of polyamine and internal standard hydrochlorides were stored at −20°C. Stock solutions of DNA (1 mg/ml in 50 mM potassium chloride) were stored at 4°C up to 4 weeks; working standards (50 μg/ml) were prepared immediately before use. DABA was purified by treating a 1 M aqueous solution with hydrochloric acid-washed charcoal (2% w/v), shaking the suspension on a vortex mixer every minute for 10 min, centrifuging (1000 ***g***) for 5 min, decanting the supernatant solution, repeating the treatment once more and filtering through Whatman 2 μm filter paper. Ether was redistilled within 24 h of use.

Equipment

A Polytron PT 10–35 homogenizer (Northern Media Supply Ltd, North Humberside) was used for preparation of tumour samples for polyamine and DNA analysis. A 'Mikro-Dismembrator' (Braun, Melsungen, F.R.G.) was used to prepare tumour samples for assay of ER binding site concentrations.

A Dri-Block DB-3 Heater (Techne (Cambridge) Ltd, Duxford, Cambridge, U.K.) was used for drying DNA precipitates.

For separation of benzoyl derivatives of polyamines a methyl octyl silyl Hypersil analytical column, 10 cm long, diameter 5 mm (Shandon Southern Products Ltd, Runcorn, Cheshire, U.K.) and a Waters Guard-PAK precolumn module charged with a Resolve CN insert (Millipore U.K. Ltd, Harrow, Middlesex, U.K.) were fitted into a Model 5000 HPLC system (Varian Associates, Walton-on-Thames, Surrey, U.K.) connected to a Varian UV-100 Detector set at 235 nm and a Varian 4270 Integrator. Samples were injected via a Rheodyne 7125 syringe loading injector fitted with a 50 μl sample loop.

A Model 3000 Fluorescence Spectrophotometer (Perkin Elmer Ltd, Beaconsfield, Buckinghamshire, U.K.) was used for DNA analysis using a quartz cell of 1 ml capacity, excitation slit 10 nm, excitation wavelength 400 nm, emission slit 5 nm and emission wavelength 504 nm.

All glass tubes and plastic ware used for the polyamine assay were rinsed with methanol before use.

Polyamine assay

The method used is based on that described by Redmond and Tseng [12]. Frozen tumour samples were thawed and dissected at 4°C. After removal of fat and necrotic tissue, replicate (2–5) pieces (15–50 mg) were homogenized (Polytron setting 7) in 2.5 ml phosphate buffer (1 mM, pH 7.4) containing internal standards (1 nmol DAH.HCl and 5 nmol DAO.HCl). Careful homogenization (three times 5 s alternated with cooling in ice for 30 s) minimized local heating. Homogenates were treated with 250 μl TCA (100% w/v) and the mixtures cooled at 4°C for 30 min, centrifuged (2500 ***g***) for 40 min and the supernatant solutions transferred to glass-stoppered test-tubes. The precipitates were kept at 4°C for subsequent DNA assay. The solutions containing polyamines were washed three times with redistilled ether (5 ml) followed by centrifugation (1000 ***g***, 5 min) to separate the two layers and aspiration of the ether phase. Any ether remaining in the tubes after the last wash was removed under a gentle stream of nitrogen at room temperature.

Four standard mixtures containing PUT.HCl (0.5, 1.0, 2.0 and 4.0 nmol), SPD.HCl (1.5, 3.0, 6.0 and 12.0 nmol) and SPE.HCl (2.5, 5.0, 10.0 and 20.0 nmol) in 2.5 ml phosphate buffer were set up at this stage and internal standards (DAH.HCl, 1 nmol, and DAO.HCl, 5 nmol in 50 μl) added, followed by 2.5 ml NaOH (1 M). After careful mixing, benzoyl chloride (10 μl) was added to all samples and standards and the solutions shaken on a vortex mixer for 10 s and allowed to stand for 5 min. A further 5 μl benzoyl chloride was added followed by shaking. The tubes were then placed in a water-bath at 25°C, mixed as before every 10 min for 30 min, and allowed to stand for another 30 min. Saturated sodium chloride solution (35 g/100 ml water, 3 ml) was added and the solutions mixed on a vortex mixer followed by extraction of the benzoylated derivatives with redistilled ether (3 ml) and centrifugation. The ether extracts were transferred to clean tubes, taking care not to pick up any aqueous phase, and the ether removed under nitrogen at room temperature. The residues were dissolved in 150 μl of HPLC solvent (methanol : water, 55 : 45) and left, with tubes tightly capped, at room temperature for 12–24 h prior to injection.

Twenty-five microlitres (or more or less, depending on polyamine concentration) of samples and standard mixtures were injected every 25 min, the HPLC solvent, sonicated before use, being run at a rate of 1 ml/min. This produced clean separations of polyamines and internal standards (Fig. 1). Successful application of this method requires attention to detail and extreme cleanliness. Results were expressed per unit wet weight of tissue or per unit weight of DNA.

DNA assay

The method used is an adaptation of that of Kissane and Robins [13]. The TCA precipitates

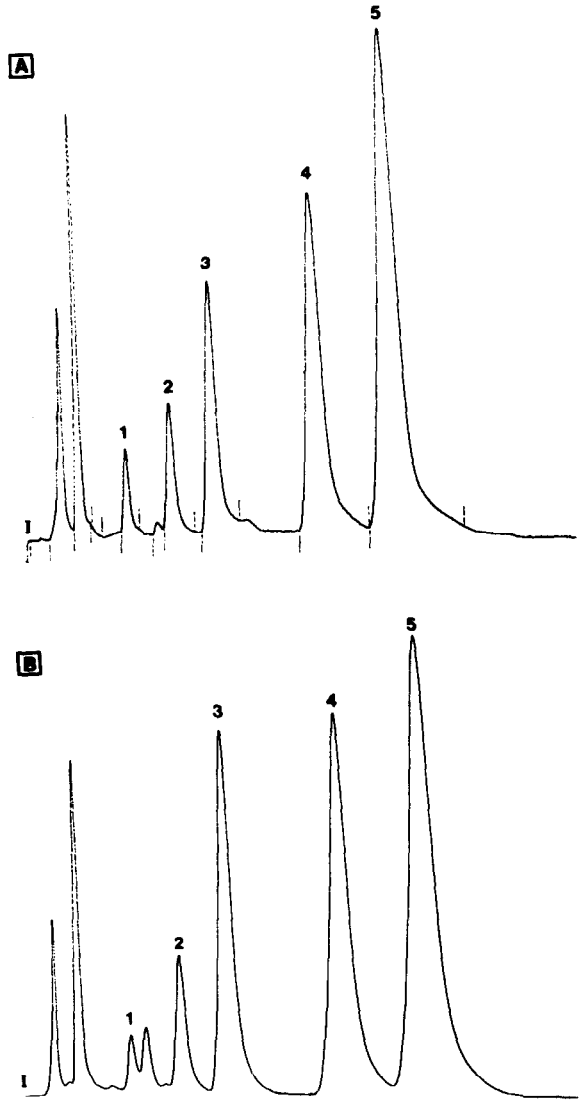

Fig. 1. HPLC tracings from two tumours. (A) Typical tracing of benzoylated derivatives. Only PUT, SPD and SPE were measurable. (B) Tracings for a minority of tumours show an additional peak. I, injection; 1, PUT; 2, DAH; 3, SPD; 4, DAO; 5, SPE.

were shaken with ethanol (0.5 ml) on a vortex mixer and a further 1 ml ethanol was added to wash down any precipitate adhering to the sides of the tubes. The tubes were heated at 60°C on a Dri-Block for 15–20 min, cooled, centrifuged (2500 ***g***, 30 min) and the alcohol wash discarded. TCA (5% w/v, 1 ml) was added to each tube.

A blank and standards containing 2.5, 5.0, 10.0 and 20.0 μg DNA were made up to 500 μl with water and TCA (10% w/v, 500 μl) was added. Samples and standards were heated at 90°C for 30 min, during which time the precipitate was broken up by drawing up and down a clean glass Pasteur pipette.

The solutions were washed twice with ether (4 ml) followed by centrifugation to separate the two phases. Any remaining ether was allowed to evaporate overnight at room temperature. Two hundred microlitres (one-fifth) of each standard and aliquots corresponding to 1 mg of tissue were dried in a vacuum oven at 55°C for approximately 1 h. Purified DABA solution (100 μl) was added to each tube, the solutions mixed and the tubes heated in the DriBlock at 60°C for 30 min. Hydrochloric acid (1 M, 750 μl) was added, the tubes shaken and the fluorescence measured within 10 min of adding the acid.

Assay of oestrogen receptor binding sites

A single saturating dose, dextran-coated charcoal technique [14] was used. The protein content of tumour supernatants was estimated by the method of Lowry *et al.* [15]. Concentrations of ER binding sites were expressed as fmol/mg protein.

Statistical methods

Within-batch and within-tumour variances were calculated from

$$\frac{\sum_{i=1}^{N}\left[\sum_{j=1}^{n_i} x_{ij}^2 - (\sum x_{ij})^2/n_i\right]}{\sum n_i - N}$$

where N is the number of tumours, n_i is the number of replicates analysed of the ith tumour sample, x_{ij} is the jth result for the ith tumour, and n, the total number of analyses, is n_i.

Mean values were compared using Student's t test.

RESULTS

Evaluation of analytical techniques

Most tumours contained peaks corresponding to PUT, SPD and SPE only (Fig. 1(A)), but a further peak (possibly cadaverine) was present in some (Fig. 1(B)). This component, when present in appreciable quantity, could interfere with the estimation of putrescine giving rise to overestimates.

A few tumour extracts contained an impurity which was eluted just before DAH and could affect is peak height. Therefore a second internal standard (DAO) was used. In most cases similar results were, however, obtained with DAH and DAO.

Estimates of within-batch precision of polyamine assays, calculated from analyses of tumour homogenates (Table 1) indicate considerable variation at low concentrations. Between-batch reproducibility was assessed by analysing samples from a pooled tumour homogenate in analytical batches over a period of 4 months. Coefficients of variation were 39.2% for PUT, mean 0.48 nmol, 11.2% for SPD, mean 1.8 nmol, 13.3% for SPE, mean 3.1 nmol ($N = 10$), and 19.3% for DNA, mean 41 μg

Table 1. Within-batch precision of polyamine assays. Replicate analyses (total number, n) peformed on N tumour homogenates. Results expressed as nmol polyamine in sample analysed. Coefficient of variation (CV) = 100 S.D./mean

Polyamine	Range	Mean	CV	N	n
Putrescine	0.13–0.43	0.28	9.9	5	12
	0.48–8.8	2.7	6.6	5	17
Spermidine	1.3–1.6	1.5	13.4	5	16
	1.7–21.0	9.9	8.0	5	13
Spermine	1.7–2.9	2.3	14.8	5	15
	3.3–22.0	11.6	5.6	5	14

Table 2. Recovery of polyamines added to tumour homogenates

	PUT		SPD		SPE	
Tumour	nmol	%	nmol	%	nmol	%
1	2	86	6	81	10	84
2	2	82	6	79	10	81
3	1	90	3	90	5	95
4	1	84	3	65	5	83

Table 3. Within-tumour variation of polyamines and DNA

	Range	Mean	CV(%)	N	n
Putrescine					
pmol/mg tissue	5.4–33	18.5	35.4	25	81
	35–125	57.9	28.6	25	81
pmol/μg DNA	4.4–14	10.0	31.3	25	81
	16–40	24.6	20.1	25	81
Spermidine					
pmol/mg tissue	20–125	89.3	26.6	25	81
	135–535	256	17.5	25	81
pmol/μg DNA	26–62	54.5	15.9	25	81
	62–127	89.9	12.2	25	81
Spermine					
pmol/mg tissue	20–289	169	33.0	25	81
	297–1042	491	18.0	25	81
pmol/mg DNA	42–102	81.5	17.3	25	81
	105–215	152	12.6	25	81
DNA					
μg/mg tissue	0.3–1.8	1.3	28.2	23	74
	1.9–14.5	4.8	24.0	23	74

($N = 11$). While the between-batch variations for SPD and SPE are consistent with within-batch data (Table 1), excessive between-batch errors were obtained for PUT.

Table 2 shows recoveries of polyamines added to four tumour homogenates. Recoveries of DNA ranged from 60 to 80%.

Polyamine, DNA and ER concentrations in tumour tissues

Polyamines were estimated in 23 malignant and 2 benign tumours, and DNA was estimated in 19 malignant and 2 benign tumours. Within-tumour variations of polyamines and DNA were studied by analysing replicate (2–5) samples in the same analytical batch (Table 3). For polyamine concentrations expressed per unit weight of tissue the coefficients of variation were well in excess of those due to analytical errors (Table 1) and were greater than those expressed per unit weight of DNA. A relationship between DNA and polyamine concentrations expressed per unit weight of tissue is suggested by the data shown in Figs 2 and 3. For patient O'H. correlation coefficients for PUT, SPD and SPE (0.936, 0.912 and 0.975, respectively) were significantly different from zero ($P < 0.01$, $P < 0.02$ and $P < 0.001$).

For patient W. no significant correlation was found for PUT. Correlation coefficients for SPD and SPE (0.894 and 0.984, respectively) were significantly different from zero ($P < 0.02$ and $P < 0.001$).

The highest concentrations of polyamines and DNA, expressed per unit weight of tissue, were found in oestrogen receptor-poor tumours (Figs 4–7). A benign fibroadenoma contained 1.9 μg DNA/mg tissue and ER binding sites of 4 fmol/mg protein. Corresponding figures for a benign mammary dysplasia tissue were 0.6 μmol/mg and 11 fmol/mg protein. For malignant tumours with ER < 10 fmol/mg cytosol protein mean concentrations ± S.D. of PUT, SPD, SPE and total polyamines were 57 ± 31, 281 ± 142, 481 ± 294 and 820 ± 437 pmol/mg tissue respectively ($N = 10$) and for ER > 10 fmol/mg protein corresponding mean concentrations were 27 ± 16, 116 ± 54, 230 ± 119 and 374 ± 168 pmol/mg tissue ($N = 13$). Mean concentrations of DNA were 5.0 ± 4.6 μg/ml tissue in malignant tumours containing ER < 10 fmol/mg protein ($N = 10$) and 1.7 ± 0.9 μg/mg tissue in those containing ER > 10 fmol/mg protein ($N = 11$). The differences between ER-poor and ER-rich tumours were statistically significant ($P < 0.01$ for PUT, $P < 0.001$ for SPD, $P < 0.02$ for SPE, $P < 0.001$ for total polyamines and $P < 0.05$ for DNA, Student's t test).

When polyamine concentrations were expressed per unit weight DNA, no significant differences were found between ER-poor and ER-rich malignant tumours. PUT, SPD, SPE and total polyamines were 17 ± 10, 74 ± 27, 117 ± 37 and 208 ± 65 pmol/μg DNA, respectively, for malignant tumours with ER < 10 fmol/mg protein ($N = 10$) and 18 ± 9.5, 71 ± 27, 130 ± 61 and 219 ± 81 pmol/μg DNA when ER was >10 fmol/mg protein ($N = 11$).

Ratios of SPD to SPE were similar in ER-poor and ER-rich malignant tumours. For ER < 10 fmol/mg protein the mean SPD/SPE ratio was 0.64 ± 0.18

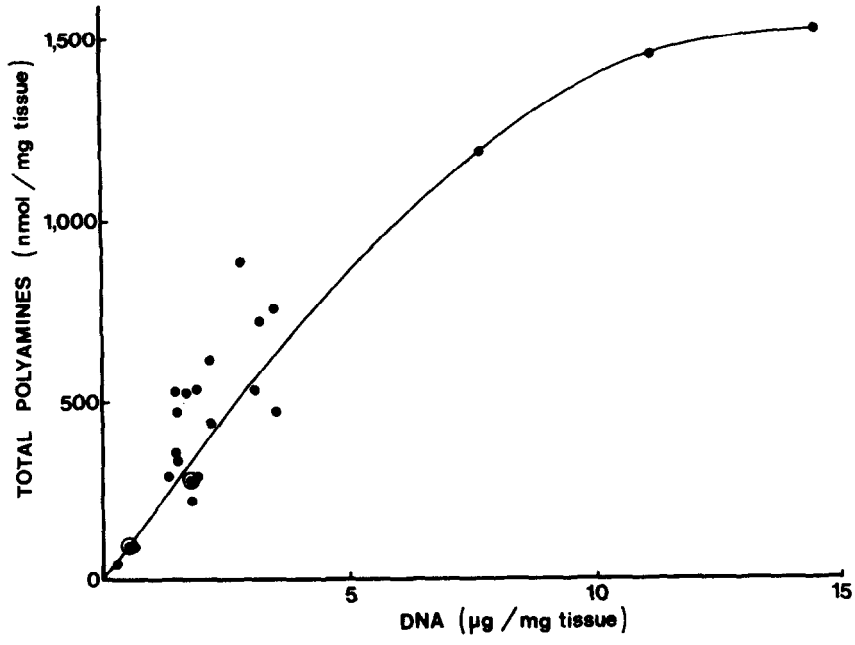

Fig. 2. Relation between total polyamine and DNA concentrations. The ringed points represent benign tumours.

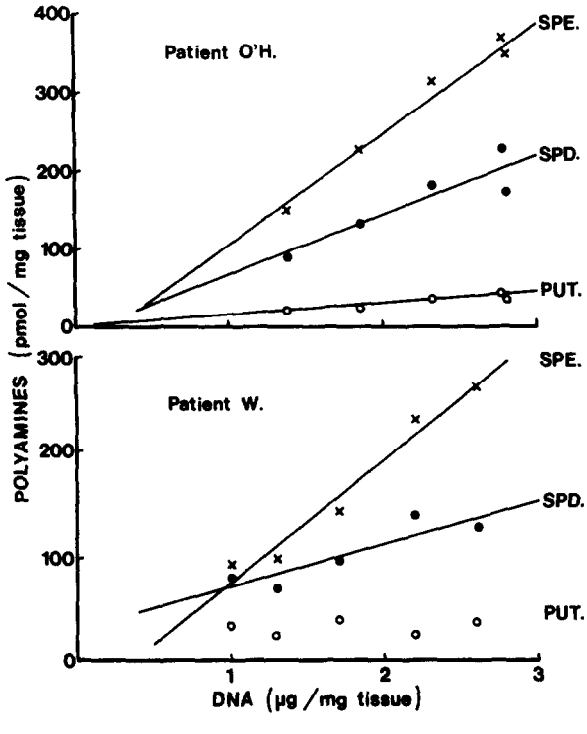

Fig. 3. Within-tumour relationships between polyamine and DNA concentrations. Replicate samples of tumour from patient O'H. (20, 21, 23, 25 *and* 29 mg) *and patient W.* (18, 18, 19, 24 *and* 33 mg) *were analysed and the results expressed per* mg *wet weight of tissue. Both tumours were malignant.*

(N = 10) and for ER > 10 fmol/mg protein it was 0.61 ± 0.31 (N = 13).

DISCUSSION

The published analytical techniques used for this study do not appear to have been assessed rigorously with respect to their accuracy when applied to tissues. Using a different method for polyamine assay Messeri *et al.* [17] reported intra- and inter-assay coefficients of variation of 5.6 and 7.7%, respectively, but did not state to what concentrations or which polyamines these parameters apply or from how many assays they were derived. Our precision of the polyamine analyses was satisfactory when samples assayed contained more than 0.5 nmol PUT, 2 nmol SPD and 3 nmol SPE. The error derived from between-batch assays for PUT seems excessive and could be due to variable overlap of small amounts of the additional component eluted close to PUT (Fig. 1) (B)). The latter, which may play a precursor role only, contributes a relatively small proportion to estimates of total polyamines. Because of lower results or shortage of tissues, nine estimates of PUT (two for non-malignant and seven for malignant tumours), six estimates of SPD (one for a non-malignant and five for malignant tumours) and five estimates of SPD (one for a non-malignant and four for malignant tumours) fell into the ranges at which CVs of 10% or more were obtained. However, all results shown in Figs 4–6 are means of three or more estimates so that the CVs for these values of PUT, SPD and SPE are reduced to at most 6, 8 and 9%, respectively, on the basis of within-batch errors or 23, 7 and 8% on the basis of between-batch variation. Recoveries of added polyamines were acceptable, although values nearer 100% would be expected for assays using internal standards. In our experience losses of the added DAH and DAO and of PUT, SPD and SPE were not identical, and this may account for recoveries short of 100%. For other studies of polyamine content of human breast tumour tissues [11, 18] no

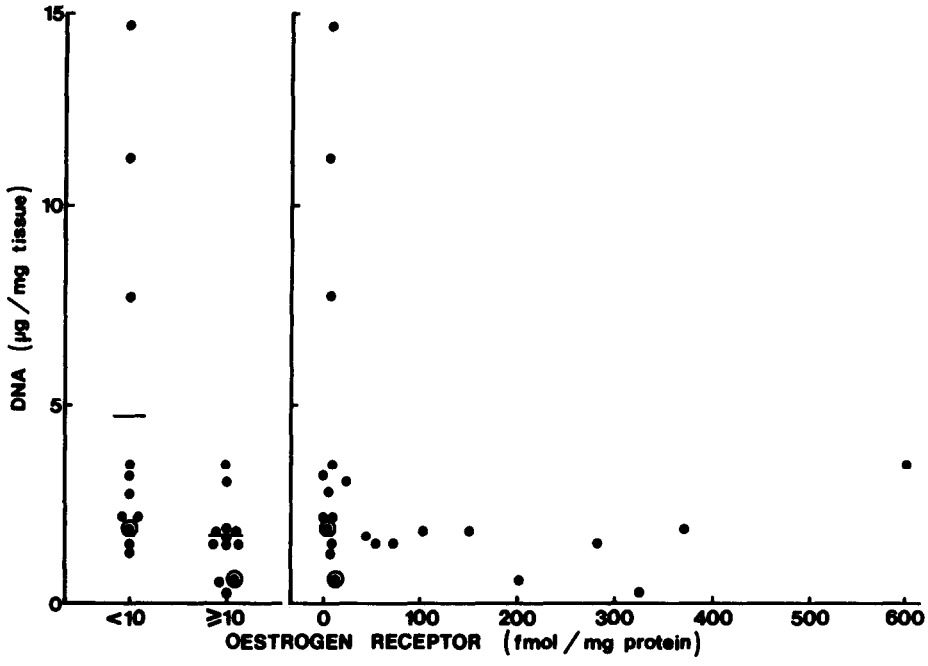

Fig. 4. Putrescine concentration and oestrogen receptor content. Horizontal lines indicate means. The ringed points represent benign tumours.

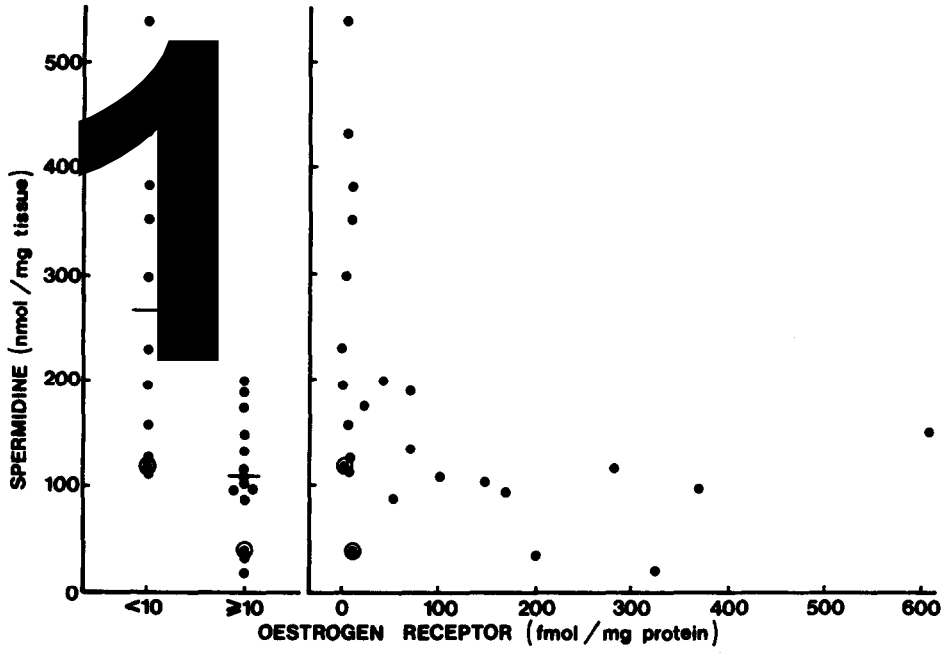

Fig. 5. Spermidine concentration and oestrogen receptor content. Horizontal lines indicate means. The ringed points represent benign tumours.

assessment of analytical reliability or within-tumour variation was presented, and the contaminant of PUT (Fig. 1 (B)) noted in a small proportion of tumours was not detected.

Our results for polyamines are higher than those reported by Kingsnorth *et al.* [11, 18]. For ER-poor tumours our values for PUT, SPD and SPE were approximately twice theirs and for ER-rich tumours only those for SPE were higher. They reported mean total polyamine levels of 336 and 232 pmol/mg tissue in ER-poor and ER-rich tumours, respectively [11], and 250 pmol/mg in further samples studied [18], while our values are 820 and 374 pmol/mg tissue in ER-poor and ER-rich tumours, respectively. It is possible that methodological losses incurred by the Scottish investigators were greater than those of our technique. While their results showed significant differences only between esti-

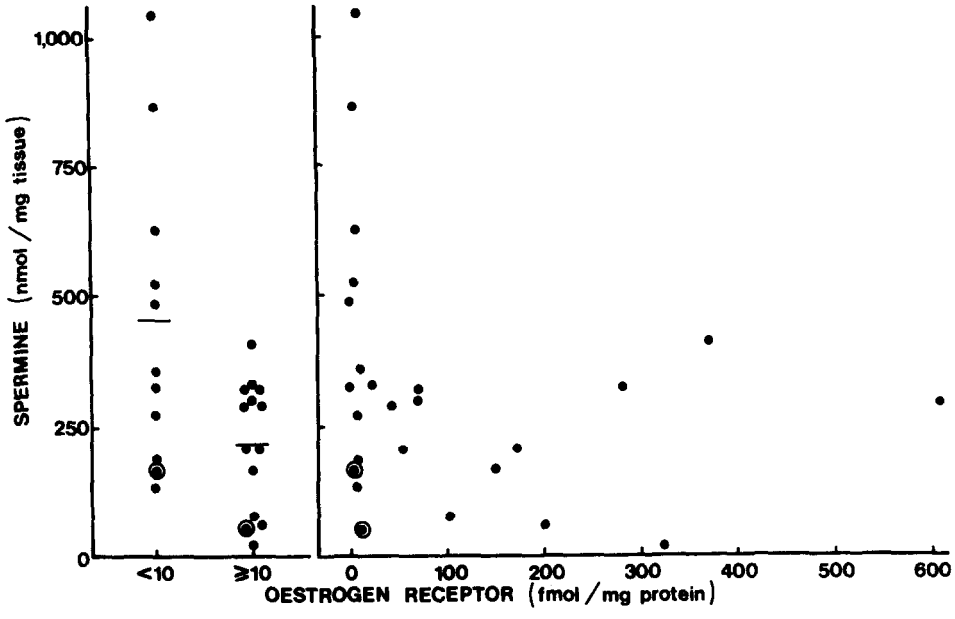

Fig. 6. Spermine concentration and oestrogen receptor content. Horizontal lines indicate means. The ringed points represent benign tumours.

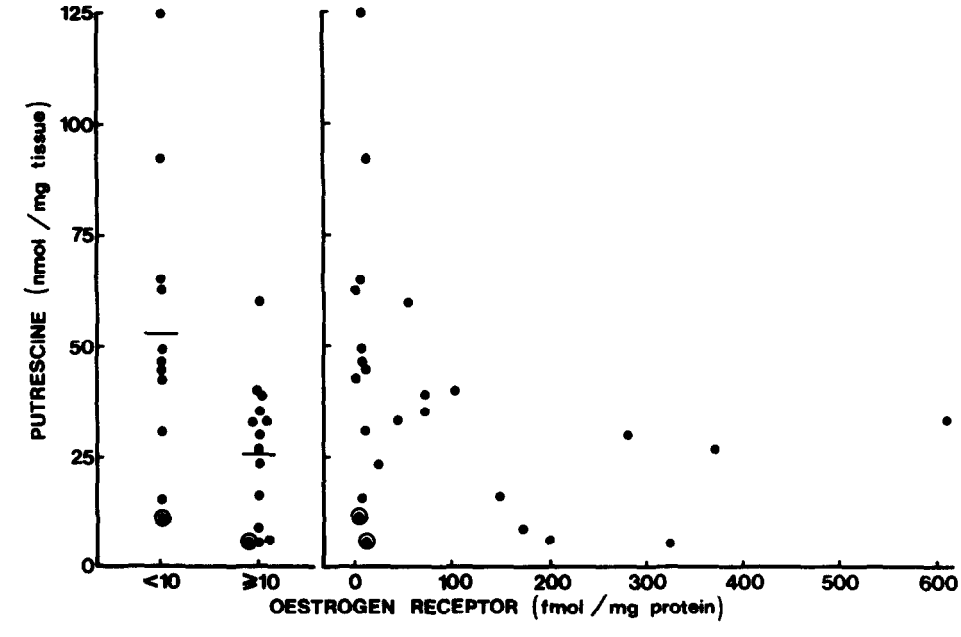

Fig. 7. DNA concentration and oestrogen receptor content. Horizontal lines indicate means. The ringed points represent benign tumours.

mates of total polyamines in ER-poor and ER-rich tumours [11], we found highly significant differences for the three individual as well as total polyamines. We thus confirm the claims that, when expressed per unit net weight of tumour, ER-poor tumours contain greater levels of polyamines than those with higher concentrations of ER binding sites. However, within-tumour variations of polyamine content thus expressed [11, 18] were considerable and it seems unlikely that such estimates can provide satisfactory prognostic indicators.

Recoveries of added DNA were lower than those reported by other investigators using similar fluorimetric assays [19, 20], and with our technique losses were probably incurred at the washing and extraction stages. The between-batch CV was high. Although others have reported CVs of 6% or less for similar methods [20–22], they did not investigate between-batch variance using quality control preparations and may have incurred greater errors than

they realized. The reliability of other methods used in studies of DNA concentrations in human breast tumour tissues [23, 24] has not been assessed.

Tumour concentrations of DNA depend on cellularity, ploidy and the proportion of cells in S and G2 phases of the cell cycle during which the DNA content of cells is greater than after mitosis. Each of these may also be related to levels of ER binding sites. The greater the cellularity of ER-rich tumours the higher their ER content. However, in a heterogeneous population of ER-rich and ER-poor tumour cells, the proportions of cell types as well as cellularity will obviously determine concentrations of ER binding sites. Two malignant tumours contained very low concentrations of DNA (0.3 and 0.6 μg/mg tissue) and high levels of ER binding sites (323 and 199 fmol/mg protein, respectively). Thus high receptor content may be associated with poor cellularity. The benign mammary dysplasia tissue, although apparently of low cellularity, contained a low but measurable level of ER binding sites, suggesting that such tissues may be responsive to oestrogen.

Cytological examination suggests relationships between ER content and ploidy. A considerable proportion of breast tumours appear to be aneuploid [25–34]. Higher proportions of ER-poor than ER-rich tumours were shown to contain aneuploid nuclei [27, 34] although this was not found in other studies [28, 32]. The proportions of cells in S phase tend to be higher in ER-poor than in ER-rich tumours [28, 32, 35, 36], and this difference, together with the possibility of increased ploidy, could lead to higher DNA concentrations in ER-poor tumours than in ER-rich ones. A significant difference was indeed demonstrated by Lidereau *et al.* [24] and is confirmed by our results. However, there is poor agreement with regard to estimates of breast tumour DNA content obtained by different analytical techniques. Deshpande *et al.* [23], using the method of Burton [37], found average values between 2 and 3 μg/mg tissue for different patient subgroups while Lidereau *et al.* [24] using u.v. absorption reported means of 1.2 μg/mg tissue in ER-rich and 1.6 μg/ml in ER-poor tumours. We found corresponding levels of 1.7 and 5.0 μg/mg, respectively, by means of a fluorimetric method, for which recoveries of added DNA were examined. It is possible that pulverization, used to break up the tissues [24], has led to destruction of some nuclei and loss of DNA to the 'cytosol' fraction.

Baserga [38] has estimated that 1 mg tissue contains roughly 5×10^5 cells and that a diploid somatic mammalian cell contains 6 pg DNA. Using these figures one arrives at a tissue DNA concentration of 3 μg/mg. Surprisingly, a limit of 0.5 μg/mg tissue has been set for adequate cellularity in the assay of hormone receptors in drill biopsy specimens [39]. This would seem to be too low and may lead to classification as receptor negative of tissues containing some intracellular ER binding sites but a low proportion of tumour cells. While a low level of DNA indicates poor cellularity, concentrations considerably greater than 3 μg/mg suggest relatively high proportions of cells in S or M and G2 phases of the cell cycle or of aneuploid cells. A combination of low cellularity and high ploidy could result in 'normal' DNA concentrations, so that on theoretical grounds this parameter would be of doubtful value in relation to prognostic indicators.

It is not surprising, therefore, that estimation of tumour DNA levels did not appear to serve a useful purpose from a clinical point of view [23]. In the present study tumour DNA concentrations ranged from less than 1 μg/mg to 12 μg/mg or more (Fig. 7), suggesting poor cellularity in some and high cellularity and ploidy in other tumours. All the ER-poor tumours contained 1.5 μg DNA/mg tissue or more, but if any of these tissues had a high proportion of high ploidy cells, cellularity could be poor and cellular ER content higher than estimates led one to believe. Such tissues could constitute 'ER-negative' hormone responsive tumours.

Positive correlation between DNA and polyamine content in human breast tumours may result from variations in tissue cellularity, from polyamine function in DNA synthesis, from chemical association of polyamines with DNA or from combinations of these. Tissue concentrations of PUT, SPD and SPE depend on both their production and catabolism and their action is related to cell proliferation and thus to DNA replication [1, 2]. Polyamine deprivation can result in tumour cell arrest in G1 or G2 phases [40] and thus growth inhibition. While polyamine action can shorten the S phase of the cell cycle [41] and hence progression to mitosis, this may or may not be associated with an increase in the growth fraction and thus high tumour DNA content. Therefore, polyamine action per se does not necessarily explain a relationship to DNA concentrations. Chemical association between intracellular DNA and polyamines, on the other hand, could result in a positive correlation between tissue concentrations of these components. Tumours with high growth fractions and hence a high proportion of cells in S phase at any time would contain more PUT, SPD and SPE per unit wet weight as a result of higher DNA concentration than those with lower growth fractions.

In our study, estimates of DNA and polyamines were obtained from the same pieces of tissue, while separate samples were used for assays of ER binding sites. In spite of this, highly significant differences were obtained for polyamine as well as DNA content between ER-rich and ER-poor tumours. Since the highest polyamine and DNA levels were associated

with low levels of ER binding sites, the relationship was not due to variations in tissue cellularity. The high polyamine and DNA contents of ER-poor tumours are unlikely to be a result of oestrogen stimulation. Hormone-independent production of growth factors by tumour cells [42] and autocrine or paracrine stimulatory action of these factors on polyamine synthesis and replication may result in higher levels of polyamines and DNA than those achieved by hormonally controlled processes.

High SPD/SPE ratios and a correlation between these and tumour growth rates have been reported for malignant liver tumours [43]. Since ER-poor breast tumours have been claimed to exhibit a poor prognosis [44] and may possibly proliferate faster than ER-rich tumours, concentrations of ER binding sites and SPD/SPE ratios might show an inverse relationship. However, mean SPD/SPE ratios were similar in ER-rich and ER-poor tumour tissues, an observation which is in agreement with a recent report [17].

It would appear that in human breast tumours there is a positive correlation between tissue content of DNA and all three polyamines and an inverse relation between ER binding site concentrations and DNA, PUT, SPD, SPE and total polyamine levels. In ER-poor tumours factors other than oestrogen may raise polyamine as well as DNA content. Since only one-half or fewer ER-poor tumours had DNA and polyamine levels above the range for ER-rich tumours, it is possible that subgroups exist which differ with respect to the nature of agents controlling DNA and polyamine levels or to responses to a single agent. ER-rich tumours showed a wide variation in polyamine and DNA levels. Although this must in part be due to variations in cellularity and ploidy, it may also indicate differences between tumours with regard to the presence of substances controlling both DNA and polyamine levels—such as oestrogen, other hormones or polypeptide growth factors—or to responsiveness to these agents.

In conclusion, while within-tumour estimates of polyamine and DNA concentrations appear to be too variable to provide useful parameters in relation to prognosis and clinical management of breast cancer, elucidation of interrelationships between these substances and the mechanisms by which their concentrations are controlled seems essential to an understanding of the between-patient variations in the progression of the disease.

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