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AROMATASE ACTIVITY IN BREAST TISSUE

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Summary—Aromatase activity may be detected both in breast adipose tissue and breast cancer in levels similar to or greater than those in other peripheral tissues. Factors influencing such local biosynthesis have been sought. Of 247 primary breast cancers investigated, 178 showed evidence of oestrogen biosynthesis. No significant relationships were found between either the presence or levels of activity and tumour histopathology, patient characteristics (such as age and menopausal status), disease stage and prognosis (determined by disease-free interval and survival after primary treatment). Aromatase was more likely to be found in cellular cancers and those which were oestrogen receptor-positive, but these were not absolute associations, activity being detected in tumours with all degrees of cellularity and both receptor-positive and receptor-negative cancers. However, in a small group of patients with metastatic oestrogen receptor-positive tumours, response to the aromatase inhibitor, aminoglutethimide, seemed confined to tumours with aromatase activity. Oestrogen biosynthesis was detected in all specimens of breast adipose tissue examined. Activities were higher in fat from breast cancer patients compared with that from women with benign breast disease. In breasts with cancer, levels were higher in quadrants bearing tumour compared with those without evidence of malignancy. It is suggested that either enhanced aromatase in breast fat promotes the appearance of overt cancer or tumour factors induce aromatase in surrounding fat. Finally, although no significant correlations were detected in postmenopausal women between local aromatase activity and endogenous oestrogens in breast cancer, perfusion studies show that *in situ* oestrogen biosynthesis is primarily responsible for oestrogen levels in the majority of breast tissues. These data suggest that local aromatase activity may influence events within the breast and may be associated with the natural history and progression of certain malignancies.

INTRODUCTION

Oestrogens play an important part in breast development and in the growth of some breast cancers. The ovary is the primary source of oestrogenic hormones in premenopausal women, but after the menopause principle sites of oestrogen biosynthesis are peripheral. These tissues include skin [1], muscle [2], fat [2, 3] and in women with breast cancer, the tumour itself [4]. In the present review attention will be focused on local steroid metabolism within the breast by (a) presenting the evidence that both adipose tissue and cancers of the breast have the potential for aromatization of androgens and (b) assessing the significance of such oestrogen biosynthesis.

Investigations of aromatase in breast cancer have examined putative associations with patient and tumour characteristics; those in breast adipose tissue have centred on the potential inter-relationship with breast cancer.

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MATERIALS AND METHODS

Assay for aromatase in breast cancers

Primary tumour was obtained from patients with histologically-proven breast cancer either by biopsy or at mastectomy. Following excision, specimens were immediately placed on ice and processed at 0°C. The tumour was dissected free of extraneous material and portions (500 mg) were finely sliced and incubated for 2 h at 37°C in Krebs Ringer buffer, pH 7.4, containing an NADPH-generating system and [7α^{3}H]testosterone (22.5 μCi, 8.9 Ci/mmol). The reaction was stopped by addition of methanol and radioinert oestradiol (500 μg) was added to monitor procedural losses. Metabolites were extracted and purified by TLC [5, 6]. Characterization of oestradiol fractions was based on chemical derivative formation involving diacetates and methyl ethers and depended on (a) similar chromatographic mobility of parent oestradiol and derivatives with authentic standards and (b) consistent specific radioactivity between oestradiol and derivatives. Using these methods conversions to oestradiol $\geq 0.02\%$ are detectable

and have been considered as evidence of aromatase activity.

Assay for aromatase in breast adipose tissue

Adipose tissue was obtained from breast cancer patients treated by mastectomy or wide local excision and from patients having excision biopsies performed for benign breast conditions. Following removal of material for histological examination, tissue was processed at 0°C. Fat was carefully separated from breast parenchyma and fibrous tissue, homogenized in phosphate buffer, pH 7.4 (1:1, w/v), and centrifuged at 800 *g* for 5 min. The infranatant was recentrifuged at 100,000 *g* for 1 h and the resultant pellet (particulate fraction) was resuspended in phosphate buffer (600 μl). An aliquot (500 μl) was then added to [1β-^{3}H]Δ4-androstenedione (1 μCi, 100 nM) and an NADPH-generating system in a final volume of 1.1 ml and incubated at 37°C for 3 h. Blank incubations were performed with bovine serum albumin (1.5 mg/ml) in place of the particulate fraction. Incubations were extracted with chloroform and the aqueous phase mixed with 5% charcoal in phosphate buffer and the charcoal precipitated by centrifugation. The resultant supernatant was decanted into a counting vial containing NE260 scintillant (Nuclear Enterprises) and counted. Counts from the blank incubation were subtracted from the counts in the test system and corrected for manipulative losses. Validation has been previously reported that radioactivity in the processed aqueous phase quantitatively reflects oestrogen production [7].

RESULTS

Aromatase in breast cancer

Of 247 primary breast cancers, 178 (72%) had evidence of aromatase activity. Levels of conversion varied between 0.02 and 0.83% (median conversion = 0.08%).

Histological assessment for cellularity, grade and special features was performed on 227 of the tumours. There was a significant trend for the presence of aromatase to be associated with increasing tumour cellularity. Thus, 85% of tumours without aromatase were of low or moderate cellularity, whereas tumours with low levels of aromatase (<0.1%) were equally likely to be of low, moderate or high cellularity, and those with high levels of aromatase (≥0.1%) were mostly of high cellularity. Aromatase activity was not significantly related either qualitatively or quantitatively to any other histological feature including special subtype, grade, lymphocytic infiltration or necrosis. No significant relationship was apparent between the presence or level of aromatase and patient age, menopausal status or stage of disease as determined by either lymph node involvement or tumour clinical size. Follow-up data were available on a subgroup of 131 patients who

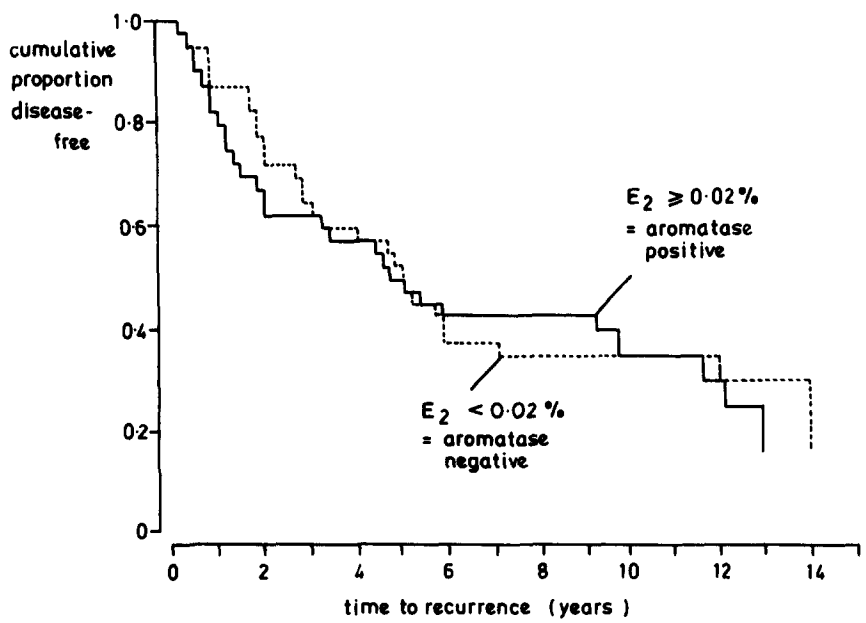

Fig. 1a. Disease-free survival curves for patients with and without aromatase activity. No significant difference between the curves by Cox analysis.

presented without clinical evidence of distant metastatic disease and were treated by some form of breast surgery. As is shown in Figs 1a and 1b, life table analyses show no significant difference in either disease free interval or survival times between tumours with and without aromatase activity.

Aromatase activity was found in both oestrogen receptor-positive and receptor-negative tumours, although there was a significant tendency for oestrogen biosynthesis to be associated with receptor-positive cancers. Forty-seven oestrogen receptor-positive tumours were obtained from patients with metastatic disease who were treated with either aminoglutethimide-hydrocortisone (23 subjects) or tamoxifen (24 patients). The relationships between response to therapy and tumour aromatase status are shown in Table 1(a, b). Thus, 11 of 18 aromatase-positive tumours responded to aminoglutethimide, whereas all 5 cancers without activity failed this treatment. This positive relationship between tumour aromatase and response to aminoglutethimide was statistically significant. A trend was also evident for aromatase-positive tumours to respond to tamoxifen but the correlation between aromatase status and response was not significant.

Endogenous oestrogens were measured in tumour extracts by radioimmunoassay after purification by column chromatography. Levels of oestradiol, oestrone and oestrone sulphate in tumours subdivided according to aromatase status are shown in Fig. 2. No significant differences were apparent in any oestrogen between tumours with and without aromatase activity, although cancers with high values of oestrogen tended to be aromatase-positive.

Table 1. The relationship between tumour aromatase status and clinical response to treatment with: (a) aminoglutethimide (1 g daily) and hydrocortisone (40 mg daily) in 23 patients with metastatic breast cancer; and (b) tamoxifen in 24 patients with metastatic breast cancer

Status	Clinical response: Partial response	Clinical response: Static or progressive disease
	*(a) Aminoglutethimide and Hydrocortisone**	
Aromatase-positive	11	7
Aromatase-negative	0	5
	*(b) Tamoxifen***	
Aromatase-positive	9	4
Aromatase-negative	5	6

*$P = 0.047$ (Fisher's exact test); **$P = 0.45$ (Fisher's exact test).

Aromatase in breast adipose tissue

Aromatase activity was detected in all specimens of mammary adipose tissue examined, levels varying from 3 to 114 fmol oestrogen equiv/mg particulate protein/h (median value 20 units).

In a comparison of adipose tissue from women with either benign or malignant breast disease [8], significantly higher aromatase activity was found in fat from patients with breast cancer. Although there was a large range of values and considerable overlap in activity

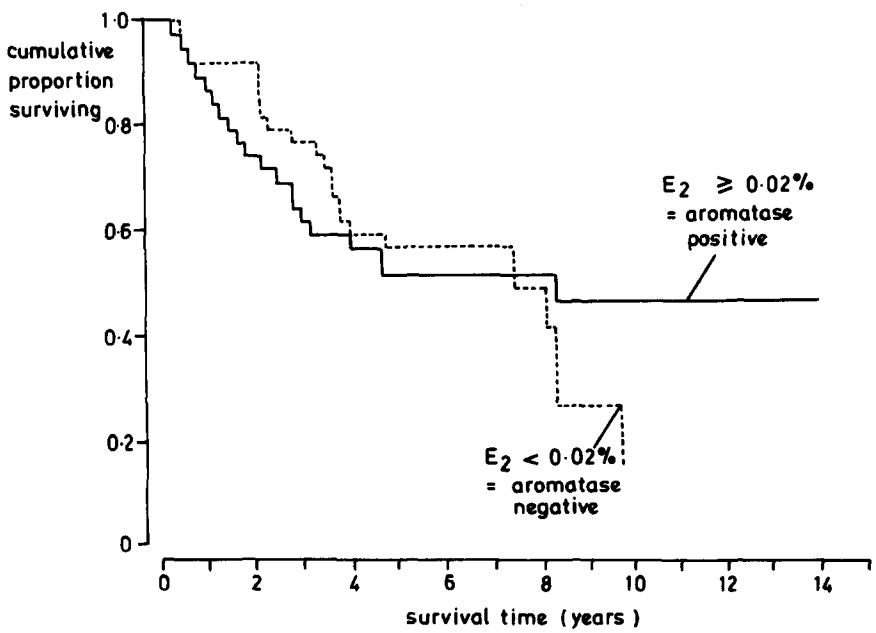

Fig. 1b. Actual survival curves for patients with and without aromatase activity. No significant difference between the curves by Cox analysis.

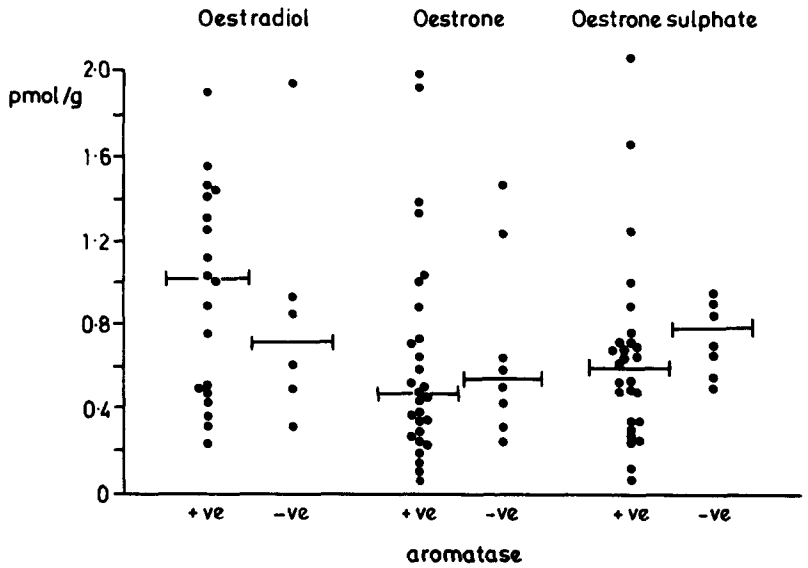

Fig. 2. Levels of oestradiol, oestrone and oestrone sulphate in tumours with and without aromatase activity. Bars represent median values.

between the two groups, the median value in the cancer group (27 units) was over 2-fold higher than that of the benign group (12 units).

In order to assess the variation in aromatase activity in adipose tissue throughout individual breasts, oestrogen biosynthesis was measured in tissue obtained from each quadrant of 12 consecutive mastectomy specimens, patients being treated for cancer of the breasts. Considerable variation in levels of activity was observed between adipose tissue from different breasts and between different quadrants of the same breast [9]. The latter variation was as great as 5-fold and had some anatomical basis (see Fig. 3). Thus, the upper outer quadrant had the highest activity in 7 of the 12 specimens and was never the site of lowest activity. In contrast, the lower inner quadrant was never the source of the highest activity and possessed the lowest activity in 5 cases. This correlation between aromatase and anatomical derivation of tissue within the breast was statistically significant ($P < 0.05$). However, as is also shown in Fig. 3, these breasts were examined clinically and histologically for the presence of cancer so that the relationship between aromatase activity in breast

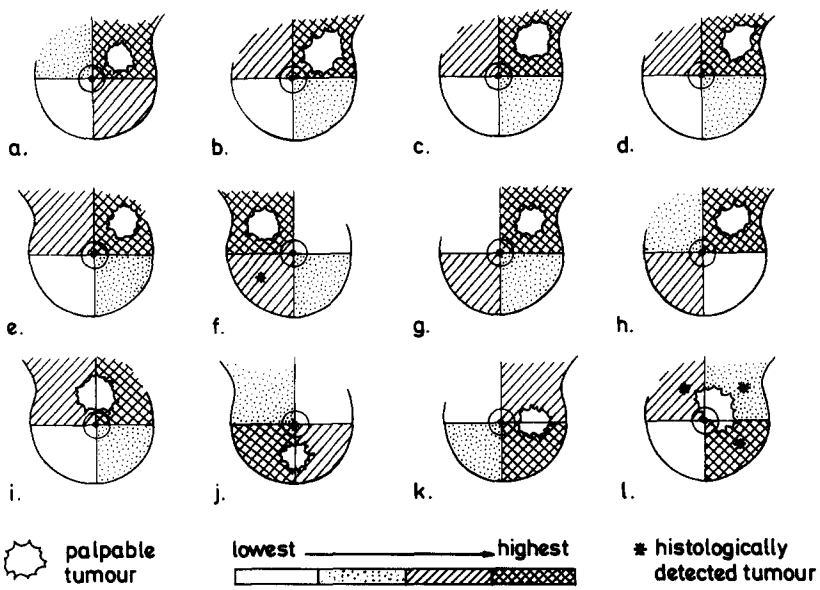

Fig. 3. The relationship between aromatase activity and tumour location in the breasts of 12 women with breast cancer, relative levels of activity are represented diagrammatically by shading.

fat and tumour site could be studied. In all 12 mastectomy specimens the quadrant displaying the highest level of aromatase was involved with tumour, whereas the quadrant with the lowest aromatase activity never contained tumour. Furthermore, where a tumour occupied more than one quadrant, these quadrants always had higher aromatase activity than quadrants which were evidently free of tumour. The relationship between tumour presence and level of aromatase was absolute and statistically significant ($P < 0.001$ by Wilcoxon signed rank test). Multiple regression analysis showed that after correction for tumour presence, the correlation between aromatase activity and anatomical derivation of tissue no longer was of statistical significance.

In an effort to determine whether enhanced aromatase activity in adipose tissue from patients with breast cancer predated appearance of overt malignancy, levels were compared in mammary fat obtained from patients with benign breast disease who either had or had not a first-degree relative with breast cancer. No significant difference in aromatase activity was observed between the groups (Fig. 4). A similar comparison in patients with breast cancer again showed that family history of cancer did not significantly influence aromatase activity in mammary fat (Fig. 4). However, levels were higher in the cancer patients compared with those with benign disease, irrespective of family history.

As it is possible that tumour factors are capable of inducing aromatase activity in surrounding fat the effects of adding a tumour extract to cultured cells derived from adipose tissue have been examined. To date, only one experiment has been performed in which adipose tissue and tumour have been obtained from the same patient. A fibroblast cell line was derived from the breast fat and studied in its 4th passage. Aromatase activity was demonstrable in particulate fractions from the cell line (Fig. 5). Addition of dexamethasone for 48 h in culture markedly induced levels of aromatase. Coculture of a homogenate (10%) of the tumour also influenced aromatase, inducing activity in the absence of dexamethasone and being inhibitory in its presence. Interestingly these effects were similar to those produced by including epidermal growth factor in parallel cultures.

DISCUSSION

Evidence has been presented that both mammary fat and the majority of breast cancers have the potential to synthesize oestrogen *in vitro*. Biosynthetic activity was comparable to that found in other peripheral tissue and if reflected *in vivo* would represent pmol amounts of oestrogen being produced locally within the breast.

The fundamental issue which requires to be resolved is whether such local biosynthesis is capable of eliciting oestrogenic responses

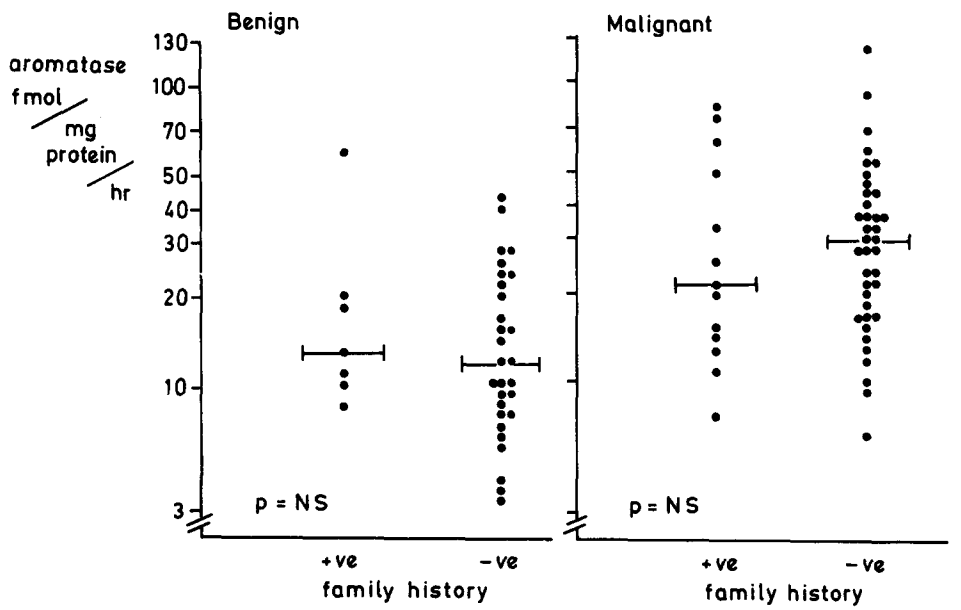

Fig. 4. Aromatase activity in breast adipose tissue from women with and without a family history of breast cancer, the tissue being obtained from patients undergoing surgery for either benign or malignant breast disease. Bar represents median values.

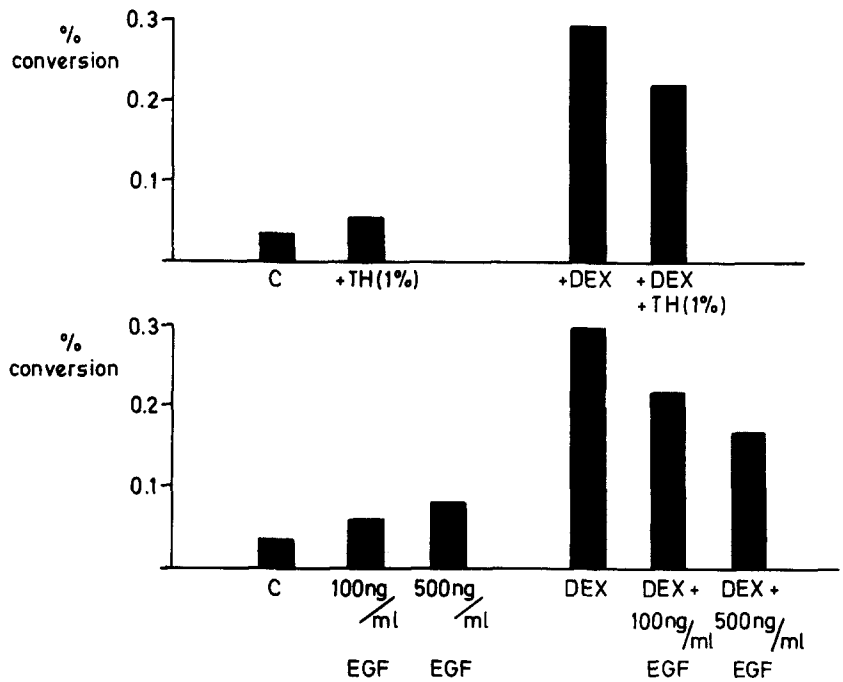

Fig. 5. Aromatase activity (expressed as percentage conversion of androstenedione to oestrogen) in human breast fat fibroblasts cultured in the absence of hormone additions (C), with a homogenate (1%) of breast cancer (TH), with dexamethazone (DEX), with both dexamethazone and the breast cancer homogenate (DEX + TH), with varying concentrations of epidermal growth factor (EGF) and with both dexamethazone and epidermal growth factor (DEX + EGF).

within the breast and, in particular maintaining oestrogen-dependent growth of breast cancers. To address this question in respect of aromatase activity in breast cancers themselves, potential for oestrogen biosynthesis has been related to tumour and patient characteristics.

The positive correlation between both presence and level of aromatase activity and tumour cellularity strongly suggests that cancer cells are the principal site of aromatase activity in tumour biopsies. This would be consistent with the observation that although aromatase activity has been detected in non-malignant compartments of the breast [8, 10], levels are invariably lower than in breast cancers [11]. Nevertheless, tumours with low cellularity are capable of displaying high levels of aromatase and some highly cellular tumours fail to synthesize oestrogens (about 15% of each population), suggesting that other factors influence aromatase activity within breast tumours. Other pathological characteristics of the tumour seem to be relatively inconsequential, no significant correlations being detected between aromatase activity and histological grade, special subtypes and features such as necrosis and lymphocytic infiltration.

Aromatase activity in other peripheral tissues has been reported to increase with age [12] but no such relationship was found with tumour oestrogen biosynthesis in the present studies. Neither was there a significant difference between levels in tumours from pre- and postmenopausal women.

In view of the lack of association between tumour aromatase and prognostic factors such as lymph node involvement and tumour size, it is not surprising that the clinical behaviour (in terms of disease-free interval and survival times) after primary surgical treatment of aromatase-positive and aromatase-negative tumours was similar in patients presenting without evidence of distant metastasis. In terms of the survival data, it should be noted from Figs 1a and 1b that the early survival advantage (at 36 months) reported previously for aromatase-negative tumours [13] was not maintained with extended follow-up and emphasizes that potentially misleading conclusions may be derived by analysis of a single time-point.

Although a significant tendency was found for aromatase activity to be associated with oestrogen receptor-positive tumours, it should be stressed the oestrogen biosynthesis may be detected in both receptor-positive and receptor-negative cancers. The relevance of biosynthesis in tumours which do not possess the classical mechanism by which to process the synthesized oestrogen must be questionable. Aromatase

activity is therefore more likely to be of significance in oestrogen receptor-positive tumours and it can be asked whether oestrogen production in these cancers confers a growth advantage that may be blocked by drugs which inhibit aromatase activity. In this respect, the results relating response to aminoglutethimide to aromatase in oestrogen receptor-positive tumours may be relevant. Thus, whilst not all tumours with aromatase activity responded to treatment with the aromatase inhibitor, the regime was more likely to produce beneficial effects in this group and no tumour without aromatase activity responded to aminoglutethimide. These results do not necessarily prove that tumour biosynthesis has provided the oestrogen required for continued growth. Tumour aromatase activity may simply reflect levels of biosynthesis in other peripheral tissues or be a marker of hormone sensitivity. In this respect it is interesting that the relationship between tumour aromatase and response to tamoxifen is less strong than that for aminoglutethimide—however, it should be emphasized that the number of patients studied is limited so that definitive conclusions can not be reached.

The lack of association between tumour aromatase status and levels of any individual oestrogen does not provide immediate support for the concept that local biosynthesis is responsible for endogenous oestrogens. However, it may be relevant that tumours with high levels of oestrogen were usually aromatase-positive. It is also possible that *in vitro* assays of enzyme activity may not precisely predict events *in vivo*. In this respect the infusion experiments of the type performed by James and his colleagues are particularly informative [14]. These studies suggest that some form of local oestrogen biosynthesis is primarily responsible for oestrogens within the majority but not all breast cancers. If in certain tumours, oestrogens are largely derived from plasma sources, this would in part explain the lack of correlation between *in vitro* measurements of tumour aromatase and endogenous oestrogen. Additionally, the infusion experiments do not identify the site of local biosynthesis within the breast which could be primarily in adipose tissue rather than the tumour. For this and other reasons it was of interest to assess aromatase activity in mammary adipose tissue and investigate its relationship to breast cancer.

The finding that mammary fat from patients with breast cancer has higher aromatase activity than that from women with benign breast disease is not completely consistent with other reports [3, 10, 15] but, in general, these studies have investigated small numbers of patients. The only other data [10] encompassing adequate numbers used the "soluble" fraction from adipose tissue (which is not the subcellular fraction with the highest aromatase content) and detected aromatase activity in 82% of cancer cells compared with only 50% of those with benign conditions. This again suggests a trend towards enhanced aromatase activity in adipose tissue from breast cancer patients.

The variation in aromatase in different quadrants of the same breast also showed that increased activity was particularly associated with areas in which tumour was identified either clinically or histologically. The possibility that increased levels of activity are due to microscopic deposits of tumour (which show higher aromatase than adipose tissue) cannot be excluded but samples of fat adjacent to those taken for aromatase assays did not show gross tumour involvement.

Two major explanations exist for increased levels of aromatase adipose tissue from breast cancer patients: (i) that inherently high activity gives rise to elevated local levels of oestrogen which encourage malignant growth at that site; or (ii) that tumours secrete factors into their local environment which either induce or stimulate aromatase activity.

If enhanced aromatase precedes and promotes the appearance of breast cancer, then it might be expected that breasts at increased risk of malignancy might show higher levels of oestrogen biosynthesis than those at low risks. However, the study of mammary adipose tissue obtained from patients with and without a family history of breast cancer failed to reveal any differences in aromatase activity.

It seems more likely therefore that the presence of a breast cancer might induce aromatase activity in adjacent adipose tissue. Although anecdotal, the experiment in which a tumour homogenate influenced aromatase in cultured fibroblasts from mammary adipose tissue of the same patient would be compatible with this concept. Similar data has also been reported by others [16]. Interestingly, epidermal growth factor was able to produce similar effects and it is known that other growth factors and cytokines, some of which may be secreted by breast cancer cell lines [17], may influence aromatase in fat [18]. These results point to the potential

for paracrine communication between adipose tissue and breast cancers.

Whilst not definitive as individual observations, in combination results of steroid perfusion studies, the association between tumour aromatase and response to aminoglutethimide and the relationship between aromatase in mammary fat and the presence of breast cancer, suggest that local oestrogen biosynthesis may influence events within the breast—particularly in postmenopausal women in whom the ovary is no longer the primary source of oestrogenic hormones. If this is the case, it will be important to define the factors which regulate aromatase activity in the breast. This could both elucidate the processes underlying hormone-associated abnormalities and provide alternative methods of treating such conditions.

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