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Aromatase, 17β-Hydroxysteroid Dehydrogenase and Intratissular Sex Hormone Concentrations in Cancerous and Normal Glandular Breast Tissue in Postmenopausal Women

A. VERMEULEN,*‡ J. P. DESLYPERE,* R. PARIDAENS,† G. LECLERCQ,† F. ROY† and J. C. HEUSON†

**Department of Endocrinology, Academic Hospital, State University of Ghent, De Pintelaan, 185 B 9000 Ghent, Belgium and †Clinique et Laboratoire de Cancérologie Mammaire, Service de Médecine et Laboratoire d'Investigation, Clinique H. J. Tagnon, Institut J. Bordet, Centre des Tumeurs de l'Université Libre de Bruxelles 1, rue Héger Bordet B 1000 Brussels, Belgium*

Abstract—*In a study of the origin of estrogens in patients with breast cancer, the concentrations of estrogens and their androgen precursors, and aromatase and 17β-hydroxysteroid dehydrogenase (E_2DH) activities were determined in normal glandular and cancerous breast tissue. The correlation between tissue estrogens, precursor concentrations, enzyme activities and plasma levels and/or receptor status were calculated. In both normal glandular and carcinomatous breast tissue, the concentrations of androstenedione (A), dehydroepiandrosterone (DHEA), 5 androstene-3β, 17β-diol (5-Adiol), estrone (E_1), estradiol (E_2) and progesterone (P) were significantly higher than plasma concentrations. While testosterone (T) concentrations were similar, dehydroepiandrosterone (DHCA) and estrone sulphate (E_1S) concentrations were lower in tissue than in plasma. In carcinomatous tissue androgen concentrations were lower, but estrogen concentrations were higher than in glandular breast tissue. Estradiol (E_2) concentration was positively correlated with the receptor concentration with the mean E_2 concentration corresponding to an estimated receptor occupancy of about 25%, probably sufficient for a submaximal biological response. Aromatase and E_2DH ($E_2 \rightarrow E_1$) activities were observed in all breast cancer and glandular breast tissues, activities being higher in carcinoma than in glandular breast tissues; nevertheless, aromatase activity accounts probably only for a small fraction of tissue estrogen concentration. E_2DH, but not aromatase activity, was significantly higher in estrogen receptor positive than in estrogen receptor negative tissues and was negatively correlated with tissue dehydroepiandrosterone (DHEA) and its sulphate (DHEAS) concentration; the latter two steroids are non competitive inhibitors of E_2DH which inactivates E_2 to E_1. This effect of DHEA (S) may constitute a mechanism by which these androgens stimulate cancer growth and a rationale (besides suppression of estrogen precursors) for medical or surgical adrenalectomy in hormone sensitive metastatic mammary cancer. E_2DH activity might constitute an additional marker of hormone dependency of mammary cancer.*

INTRODUCTION

IT IS WELL documented that sex steroids play a role in the development of mammary cancer and exposure to estrogens is considered to be an important factor in progression of the disease (for review see Thomas, 1984) [1].

It has been suggested that plasma-free estradiol levels are higher in postmenopausal women with mammary carcinoma than in controls [2, 3], whereas others [4–6] reported higher sex hormone binding globulin (TeBG) levels in estrogen receptor positive (ER+) than in estrogen receptor negative (ER–) breast cancer patients and suggested that patients with high TeBG levels, and therefore low free estradiol concentrations, may have a longer disease-free interval [6].

Intracellular estradiol can be taken up from the plasma or may be synthetized *in situ*. A number of studies have shown estrogen uptake and accumulation by human mammary carcinoma after intravenous injection of labelled estradiol [7–9] and Duvivier *et al.* [10] have shown that estradiol concentration in efferent venous blood is lower than in afferent arterial blood of the cancerous breast. Van Landeghem *et al.* [11], on the other hand, did not confirm this observation. Thus, in view of these conflicting results, and since it has been repeatedly demonstrated that estrogen concentration in mammary tissue is an order of magnitude higher than in plasma [12–14], the origin of intratumoral estrogens remains unclear. It is prob-

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‡To whom all reprint requests should be addressed.

able that estrogen uptake is not the only source of estrogens in breast tissues and that a large part might be formed *in situ*.

The present work is a contribution to the study of the origin of estrogens in mammary cancer tissues and of the factors that determine their concentrations. We therefore assayed sex hormone concentrations as well as the two main enzyme activities which contribute to local estrogen concentration, i.e. aromatase and 17β hydroxysteroid-dehydrogenase (E_2DH) in mammary cancer as well as in normal glandular breast tissue. Aromatase converts androgens (androstenedione and testosterone) into estrogens (estrone — E_1 — and estradiol — E_2 — respectively), while E_2DH converts E_2 into the less active metabolite E_1 [15, 16]. Among other factors that might affect estrogen levels in mammary tissues, we studied the correlations existing between plasma steroid levels, tissue precursor androgen concentration and estrogen receptor status. In addition we calculated the correlations of enzyme activity with receptor status and/or steroid concentrations in tissues.

MATERIALS AND METHODS

1. *Materials*

(1,2^{3}H) Testosterone (T) (TRK 162 s.a. 51 Ci/mmol); (2,4,6,7^{3}H) E_2 (TRK 322 s.a. 114 Ci/mmol); (2,4,6,7^{3}H) E_1 (TRK 321 s.a. 88 Ci/mmol); (6,7^{3}H) E_2 (TRK 125 s.a. 54 Ci/mmol); (1,2^{3}H) 5-androstene-3β,17β-diol (5-Adiol) (NET 501, s.a. 46 Ci/mmol); (7 ^{3}H) dehydroepiandrosterone (DHEA) (TRK 163 s.a. 22 Ci/mmol); (7 ^{3}H) androstenedione (NET 181 s.a. 45 Ci/mmol); (7 ^{3}H) dehydroepiandrosterone sulphate (DHEAS) (NET 121 s.a. 22.1 Ci/mmol); (6,7 ^{3}H) estrone sulphate (E_1S) (NET 203 s.a. 52 Ci/mmol); (1,2^{3}H) progesterone (P) (TRK 341 s.a. 50 Ci/mmol) (1β,2β^{3}H) androstenedione (NET 181 48.5 Ci/mmol) and (4-^{14}C)/E_1 (CFA 321 s.a. 55 mCi/mmol) were obtained either (TRK and CFA) from the Radiochemical Center (Amersham, U.K.) or (NET) from New England Nuclear (Boston, MA). Reference steroids were obtained from Steraloids Inc. (Wilton NH). Sodium azide (Merck art. 6688), $MgCl_2$ (Merck art 5833); EDTA (Titriplex-RIII Merck), nicotinamide (Sigma), NADPH (Boehringer); NAD (Boehringer); dithiothreitol (BDH); Triton X- 100 (Fluka) and other reagents used were analytical grade.

For homogenisation of the tissues, a dismembranator (Braun Melsungen F.R.G.) was used.

2. *Patients*

Mammary tissue was obtained at mastectomy for mammary cancer. None of the patients showed evidence of distant metastatic disease on clinical examination, echography or radiography and all were in good health. None had received any hormonal preparation for the last year. All were postmenopausal, that is at least 1 yr had elapsed since their last menstrual period. Plasma samples were taken just before surgery between 8 and 11 a.m. Body mass index (BMI) was calculated as body weight in kilograms divided by the square of the height in meters.

3. *Tissue preparation*

Immediately after surgical removal, normal glandular and carcinomatous mammary tissue were carefully dissected free from fat or other tissue (skin, muscle) and remaining blood was removed by repeated rinsing with phosphate buffer (pH 7.3, 0.066 M) followed by blotting with filter paper, the nature of tissue being verified by the pathologist. After dissection, tissues were kept at −60°C until analysed.

Estrogen (E_2R) and progesterone (PgR) receptor concentrations were determined in the cancerous tissues, following the recommendations of the European Organisation for Research and Treatment of Cancer (EORTC) [17] and expressed as fmol/mg protein, the latter being determined by the method of Lowry. The limit of sensitivity was set at 5 and 10 fmol/mg protein for E_2R and PgR respectively.

4. *Tissue steroid concentrations*

Testosterone (T); androstenedione (A); dehydroepiandrosterone (DHEA) and its sulphate (DHEAS); 5-androstene-3β, 17β-diol (5 Adiol); estrone (E_1), estradiol (E_2) and progesterone (P) were determined as previously described [18]. In brief, ± 500 mg of tissue, chilled in liquid nitrogen at −196°C, was pulverized with a dismembranator. The powder obtained was homogenized in 5 ml of phosphate buffer, 0.066 M, pH 7.3 and appropriate tritiated steroid tracers were added as internal standards. After brief equilibration, extraction was performed with methanol–acetone (1/1; v/v). The supernatant was evaporated under a stream of nitrogen, the residue was defatted overnight with 70% methanol and after evaporation of the methanol, the watery residue was extracted with ether. One fifth of the ether extract was purified on paper (Bush A) for separation of A, DHEA and P, whereas the remaining 4/5 were further purified on paper (Bush B3), separating E_2 from 5 Adiol and from E_1 plus T. The latter two steroids were separated on TLC (cyclohexane-ethylacetate 1 : 1, v/v). Finally the E_2 and the E_1 fractions were each purified on a Sephadex LH 20 column.

DHEAS and E_1S, in the watery residue remaining after ether extraction, were solvolysed over-

night at pH 1 [19] and 37°C, subsequently extracted with ethyl acetate and purified by paper chromatography (Bush A2 system on Whatman n°40 paper) which separates DHEA from E_1. After elution DHEA was quantified by RIA, the E_1 fraction being further purified on a LH20 column and finally quantified by RIA.

5. *Plasma steroids*

Plasma steroids were determined by specific RIA methods as previous described [20–23].

E_1S was determined after solvolysis according to the method of Hawkins and Oakey [24].

TeBG binding capacity was calculated from the free T fraction obtained by dialysis [22] assuming an association constant, $K = 1.6 \times 10^9 M^{-1}$.

6. *Aromatase activity*

Aromatase in tissue homogenates was determined according to the method of Thompson and Siiteri [25], as modified by Weisz *et al.* [26], in which tritium liberated during the aromatization of 1β-^{3}H androstenedione is used as a parameter of aromatization.

1β-^{3}H androstenedione was prepared by alkaline enolization to remove the (2-^{3}H) from (1β,2β-^{3}H) androstenedione (NET 181) [26].

This method has been validated for breast cancer tissue by Tilson-Mallett [27].

In brief, 0.2 g of homogenized tissue (Dismembranator Braun–Melsungen) was suspended in 0.5 ml of 0.066 M phosphate buffer pH 7.3, containing Triton X-100 1 μl/ml; sodium azide, 1 mg/ml; dithiothreitol 1.5 mg/ml; EDTA 0.38 mg/ml; $MgCl_2$ 1 mg/ml and nicotinamide 1,2 mg/ml (10 mM). After shaking for 1 hr at 4°C, 200 μl of the supernatant was incubated with 1 uCi 1β-^{3}H androstenedione, 0.69 μM cold androstenedione and 0.64 mM NADPH at 37°C for 1 hr. The reaction was terminated in an ice bath. 400 μl of a charcoal dextran solution was added to the incubate and after equilibrium at 4°C for 10 min, the tube was centrifuged at 800 ***g*** for 15 min and the supernatant was brought onto a charcoal/Sephadex column (l = 15 cm; i.d. = 0.8 cm) and eluted with phosphate buffer [26, 28].

On another aliquot of the tissue homogenate, protein content was determined by the method of Lowry, with albumin as the standard. Buffer blanks, treated like the tissue supernatants, were run in each assay and the radioactivity in these, which averaged 140 ± 30 (S.D.) dpm, subtracted from the counts obtained in the tissue incubation. Counts 2-fold higher than those detected in the buffer blanks were considered significant, yielding a limit of detection of 5 fmol E_1 formed/mg protein/hr. Number of moles of estrogen formed were calculated from the corrected disintegrations per minute, using the specific activity of 1β-^{3}H androstenedione in the incubate.

Results are expressed as fmol E_1/mg prot./h. All determinations were performed in triplicate.

Formation of E_1 from A was linear for the duration of the experiments (120 min) and the amount of product formed was a linear function of the amount of enzyme (tissue) incubated.

The apparent Michaelis Menten constant, determined by the method of Lineweaver and Burk [30] using triplicate measurements of the initial velocity of E_1 production by breast glandular tissue at 37°C and pH 7.3 over a range of substrate concentrations between 0.01 and 2.8 μM of A, at a single point (60 min), was 0.25 μM and the V_{max} 7.4 ± 3.7 (S.D.) fmol E_1/mg prot/hr ($n = 20$).

Routine determinations of aromatase activity were performed at a substrate concentration of 0.69 μM A ($2.7 \times K_m$).

7. *17β-hydroxysteroid dehydrogenase (E_2DH) activity*

The conversion of E_2 into E_1 was used as parameter of the 17β-dehydrogenase activity, using the method of Folkerd and James [29].

The homogenate of 1 g tissue (dismembranator) was suspended in 5.5 ml phosphate buffer pH 7.3, 0.066 M, containing Triton X-100, 1 μl/ml buffer; sodium azide 1 μg/ml; $MgCl_2$ 1mg/ml and nicotinamide 1.2 mg/ml (10 mM).

After shaking for 1 hr at 4°C, 1 ml was taken for determination of protein content (Lowry), whereas 1 ml was used for 17β-hydroxysteroid dehydrogenase activity measurement. The latter was incubated for 1 hr at 37°C with 0.2 μCi (6,7^{3}H) E_2 (purified by paper chromatography), 3.6 μM of cold E_2 and 7.5 mM NAD. Immediately after incubation, 1000 dpm of 4-^{14}C-E_1 were added as internal standard, the incubation mixture extracted with ether and purified by paper chromatography in the Bush B3 system; after elution of the E_1 zone, the latter was further purified by TLC on silica gel in the system benzene-ethyl acetate 85:15 (v/v).

Purity of the E_1 fraction was verified by constancy of $^3H/^{14}C$ ratio upon repeated cristallization, and the amount of E_1 found was expressed in pmol E_1/mg protein/hr.

The formation of E_1 from E_2 was found to be a linear function of the duration of the experiment (up to 150 min) and the amount of enzyme (tissue). The rate of formation of E_1 increased linearly with substrate concentration up to ± 2.5 μM. Optimal conversion was obtained at an NAD cofactor concentration of 7.5 mM.

The apparent Michaelis–Menten constant (K_m) for the conversion of E_2 to E_1, determined by the method of Lineweaver–Burk using triplicate measurements after incubation for 60 min at 37°C,

pH 7.3, 0.066 M, over a range of substrate concentrations between 0.02 and 5.2 μM E_2, was 1.2 μM.

Statistics

Non-parametric statistics were used: Wilcoxon signed rank test, Mann–Whitney U test, Spearman rank correlation coefficient as indicated.

RESULTS

Hormone concentration

Mean sex hormone concentrations in plasma, and in cancerous and normal glandular breast tissue are given in Table 1. DHEA, 5Adiol and DHEAS concentrations were significantly ($P < 0.05$) and P concentration marginally ($P < 0.1$) lower in the cancerous tissue than in the glandular tissue (paired observations).

Cancerous and glandular tissue concentrations (paired observations) were all positively correlated, but statistical significance was reached only for T, E_1, E_1S and DHEA ($P < 0.01$) (Table 2).

Plasma E_2 concentrations were positively correlated with BMI ($r = 0.38$; $P < 0.02$) but for E_1 and

Table 1. Steroid concentrations in plasma and in tumorous and glandular breast tissue as well as ratios of tissue to plasma concentration

	Plasma steroid concentr. (ng/ml) $n = 35$ $m \pm$ S.D.	Tumorous tissue (pg/mg prot.) $n = 50$ $m \pm$ S.D.	Glandular tissue (pg/mg prot.) $n = 14$ $m \pm$ S.D.	Tissue/plasma ratio (ng/g/ng/ml†) Tumour $n = 37$ $m \pm$ S.D.	Tissue/plasma ratio (ng/g/ng/ml†) No.Gland $n = 10$ $m \pm$ S.D.
T	0.26 ± 0.14 (0.11–0.57)‡	17.4 ± 20.6 (0.1–110)	13.1 ± 12.4 (0.3–44.2)	1.28 ± 1.19 (0.05–5.07)	1.34 ± 1.27 (0.09–3.42)
A	1.66 ± 1.10 (0.48–4.67)	263 ± 322 (10–1685)	198 ± 100 (18–412)	2.10 ± 1.57 (0.73–7.57)	5.43 ± 4.17 (1.02–14.65)
DHEA	4.28 ± 3.37 (0.68–14.43)	661 ± 723 (81–3464)	4054 ± 5368* (274–16200)	7.68 ± 8.64 (0.51–30.16)	35.63 ± 42.33** (7.13–15065)
DHEAS	406 ± 225 (103–906)	2954 ± 2878 (98–12600)	17808 ± 19510* (2997–61600)	0.42 ± 0.45 (0.02–2.01)	2.43 ± 3.12** (0.26–8.93)
5-Adiol	1.40 ± 0.94 (0.26–3.80)	91 ± 84 (2–356)	195 ± 185* (26–607)	3.95 ± 3.12 (0.42–11.18)	4.35 ± 3.85 (1.65–11.87)
E_1	0.029 ± 0.017 (0.010–0.085)	6.8 ± 13.8 (0.1–67.0)	3.9 ± 5.2 (0.1–20.0)	3.92 ± 3.51 (0.13–13.31)	3.54 ± 4.07 (0.01–13.72)
E_1S	0.32 ± 0.28 (0.02–1.05)	5.1 ± 6.9 (0.1–34.1)	0.6 ± 1.5* (0.1–1.2)	0.64 ± 0.85 (0.05–2.73)	N.D.
E_2	0.019 ± 0.008 (0.010–0.041)	8.9 ± 10.3 (0.4–49.1)	5.7 ± 4.9 (0.8–15.0)	21.49 ± 36.39 (1.40–173.00)	27.51 ± 39.34 (0.57–120.00)
P	0.47 ± 0.27 (0.04–0.21)	73 ± 97 (9–585)	138 ± 152 (41–475)	8.07 ± 8.76 (0.50–26.09)	N.D.
TeBC 10^{-8}M	3.64 = 1.82 (1.46–8.46)				
E_2R fmol/mg prot.		108 ± 179 (0–910)			
PgR fmol/mg prot.		52 ± 120 (0–609)			

* $P < 0.05$ gland vs tumour. (Wilcoxon for paired obs.)

** $P < 0.01$ gland vs tumour. (Wilcoxon for paired obs.)

† All values significantly > 1.00 ($P < 0.01$) except for DHEAS and E_1S (significantly < 1.00 ($P < 0.05$) and T)

‡ Range.

E_1S, correlation with BMI ($r = 0.22$) failed to reach statistical significance. E_1 and E_2 plasma levels were positively correlated, ($r = 0.48$, $P < 0.01$), with a mean plasma E_2/E_1 ratio of 0.40 ± 0.30 (S.D.), significantly lower ($P < 0.01$, paired Wilcoxon) than in either carcinomatous (1.10 ± 1.06 S.D.) or glandular breast tissue. We observed no correlations between plasma and tissue estrogen concentrations in either the cancerous or the glandular breast tissue but there was a positive correlation between plasma and tissue levels of A and 5-Adiol ($P < 0.01$) (Table 2).

Out of 50 cancerous breast tissues from postmenopausal women, 33 were E_2R positive (> 5 fmol/mg protein) and 17 (34%) PgR positive (only one in the absence of detectable E_2R). PgR concentration was highly significantly correlated with E_2R and weakly with tissue E_2 but not with P concentration (Table 2).

E_2 concentration in breast cancer tissue was positively correlated with E_2R concentration, but this relationship did not hold for E_1 or E_1S concentrations (Table 2).

Plasma TeBG binding capacity finally was similar in women with E_2R+ or E_2R- breast cancers, as were plasma E_2 levels.

When expressed in ng/g, all hormone concentrations in either cancerous or normal glandular tissue were significantly higher than plasma concentrations expressed in ng/ml, except for DHEAS, E_1S and T (Table 1), with the highest mean ratio for E_2.

Enzyme activities

Aromatase activity was found in all glandular and carcinomatous breast tissues examined.

Mean aromatase activity in glandular breast tissue [8.6 ± 2.9 (S.D.) fmol/mg prot/hr, $n = 11$] was significantly lower than in carcinomatous tissue [19.6 ± 10.3 (S.D.) fmol/mg prot/hr; $n = 23$]; no correlation was found between aromatase activity in either normal glandular breast or carcinomatous tissue and steroid concentration, BMI or age respectively. In carcinomatous tissue, aromatase activity was similar whether tissue was either E_2R + or E_2R − (22.1 ± 16.0 S.D. fmol/mg/hr vs. 17.7 ± 4 fmol/mg prot-hr).

E_2DH ($E_2 \rightarrow E_1$) was observed in all tissues examined. In glandular breast tissue mean E_2DH activity measured routinely at a substrate concentration of 3.6 μM E_2 was 187 ± 118 (S.D.) pmol/mg protein/hr ($n = 19$), whereas in tumorous tissue, mean E_2DH activity was 359 ± 261 (S.D.) pmol/mg prot/hr ($n = 23$) ($P < 0.01$).

E_2DH activity in tumorous ($P < 0.01$) as well as in glandular tissue ($P < 0.05$) was inversely correlated with the logarithm of the tissue DHEA concentration (Fig. 1). In tumorous tissue E_2DH activity also correlated inversely with DHEA-S concentration ($P < 0.05$) (Fig. 2). Linewear–Burk plots of data obtained from incubation studies in the presence of 2 μM of either DHEA or DHEAS indicated that the inhibition of the E_2DH activity was of the non-competitive type (Fig. 3). Mean E_2DH activity was significantly higher ($P < 0.02$)

Table 2. Correlations (Spearman rank)

	Cancerous vs. gland.breast tissue concentration $n = 14$ (paired)	Plasma levels vs. cancer tissue conc. $n = 37$	E_2R (PostM.P.) vs. cancer tissue conc. $n = 50$	E_2DH activity vs. cancer tissue conc. $n = 23$	
T	$r = 0.85$**	0.02	PgR	0.52***	ln DHEA −0.78**
DHEA	0.66**	0.05	E_2	0.47**	ln DHEAS −0.44*
DHEAS	0.40	0.21	E_1	−0.19	age 0.10
A	0.30	0.44**	E_1S	0.05	BMI −0.03
5-Adiol	0.45	0.31*	DHEA	0.02	E_2R† 0.21
E_1	0.86**	0.03	P	0.10	
E_1S	1.00**	0.18	E_2DH	0.21	
E_2	0.27	0.12			
P	0.29	0.06			

* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.
† Correlation of E_2DH with E_2R concentration in E_2R† group.

in E_2R + (n = 10) than in E_2R – (n = 11) tumorous tissues, i.e. 510 ± 108 (S.E.) pmol/mg protein/hr vs. 238 ± 19 (S.E.) pmol/mg protein/hr. Within the E_2R + group, we did not however, observe any significant correlation between E_2DH activity and E_2R concentration (Table 2).

DISCUSSION

Plasma steroid hormone concentrations in the patients with breast cancer (Table 1) are comparable to those reported in the literature [31, 32], taking into account that our group of postmenopausal women included patients in the immediate postmenopausal years and that plasma levels were not taken under basal conditions [33]: this explains the relatively broad range of values as well as the relatively high estradiol levels. The latter were similar in patients with either E_2R + or E_2R — breast tumors and plasma E_2 levels were, as expected [31], positively correlated with BMI. We could not confirm however the data of Murayama *et al.* [4–6], showing higher TeBG levels in patients with E_2R + than in patients with E_2R – tumors.

Sex hormone concentrations in breast tissue cover a wide concentration range for each steroid (Table 1). This is in accordance with all data in the literature [12–14, 34]. Breast tissue concentrations (ng/g) of sex hormones are significantly higher than plasma (ng/ml) concentrations, except for DHEAS, E_1S and T (Table 1).

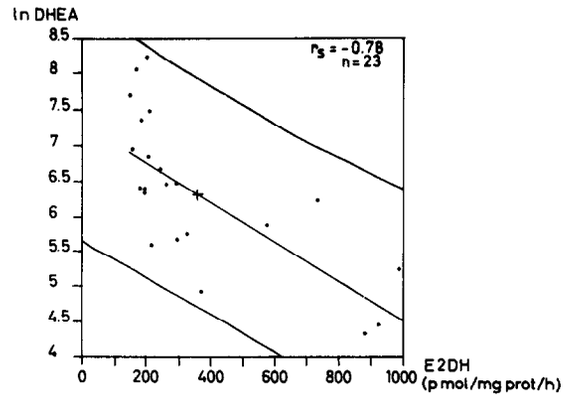

Fig. 1. Correlation between E_2DH ($E_2 \rightarrow E_1$) activity and intratissular DHEA concentration in mammary cancer tissue. (Spearman rank)

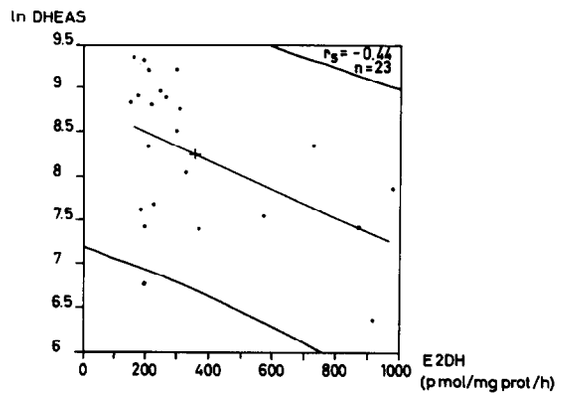

Fig. 2. Correlation between E_2DH ($E_2 \rightarrow E_1$) activity and intratissular DHEAS concentration in mammary cancer tissue. (Spearman rank)

It may be assumed that uptake from plasma is probably the major factor in this gradient for the androgens. This is also suggested by the positive significant correlation between plasma and tissue A and Adiol levels. As far as T is concerned, the absence of a tissue plasma gradient is probably the consequence of its specific binding in plasma to TeBG, whereas the absence of a positive gradient for DHEAS and E_1S is probably the consequence of the strong ionic and polar character of these steroids, rendering permeation through the cellular membrane very difficult [41, 42]. Vignon *et al.* [43] observed E_1S to be taken up by MCF7 cells, but the uptake was 7-fold lower than that of unconjugated estrogen. Similarly Verheugen *et al.* [41] observed that uterine capillary permeability was much lower for E_1S than for either E_1 or E_2. Our data are in agreement with these observations.

Concentrations of all Δ 5 steroids studied (DHEA, 5 Adiol and DHEAS) are significantly lower in carcinomatous than in normal glandular tissue (Table 1). Van Landeghem *et al.* [39], found similar concentrations in mammary cancer tissue and in normal breast tissue from 6 postmenopausal women, whereas Bonney *et al.* [34] observed higher concentrations in tumors than in normal breast tissue. However, it seems that the data of the latter workers refer to concentrations in a mixture of fat and glandular tissue, rather than in almost pure glandular tissue as in this study.

This lower concentration might indicate a more active Δ^5-dehydrogenase-isomerase activity in carcinomatous breast tissue or a defect in the uptake of these steroids in the carcinomatous cells.

Bonney *et al.* [35] as well as Vanlandeghem *et al.* [36] found estradiol concentrtaions in cancer tissue to be significantly higher than in normal glandular tissue of the same breast; our results confirm these data, although due to the wide range of values, a statistical significant difference was only found for E_1S concentration. Our data confirm the higher E_2 concentration in receptor positive than in receptor negative tumors [12, 14, 36–38]. Moreover, in distinction with Van Landeghem *et al.* [36], we found a significant positive correlation between E_2 and E_2R concentrations ($P < 0.01$) (Table 2), with a mean E_2 concentration of 10.8 ± 2.1 pg/mg protein in E_2R positive against 5.3 ± 1.0 pg/mg protein in E_2R negative tumors ($P < 0.05$). As expected we observed significant correlation between PgR concentration and E_2R concentration (Table 2): indeed, PgR concentration is considered to be a

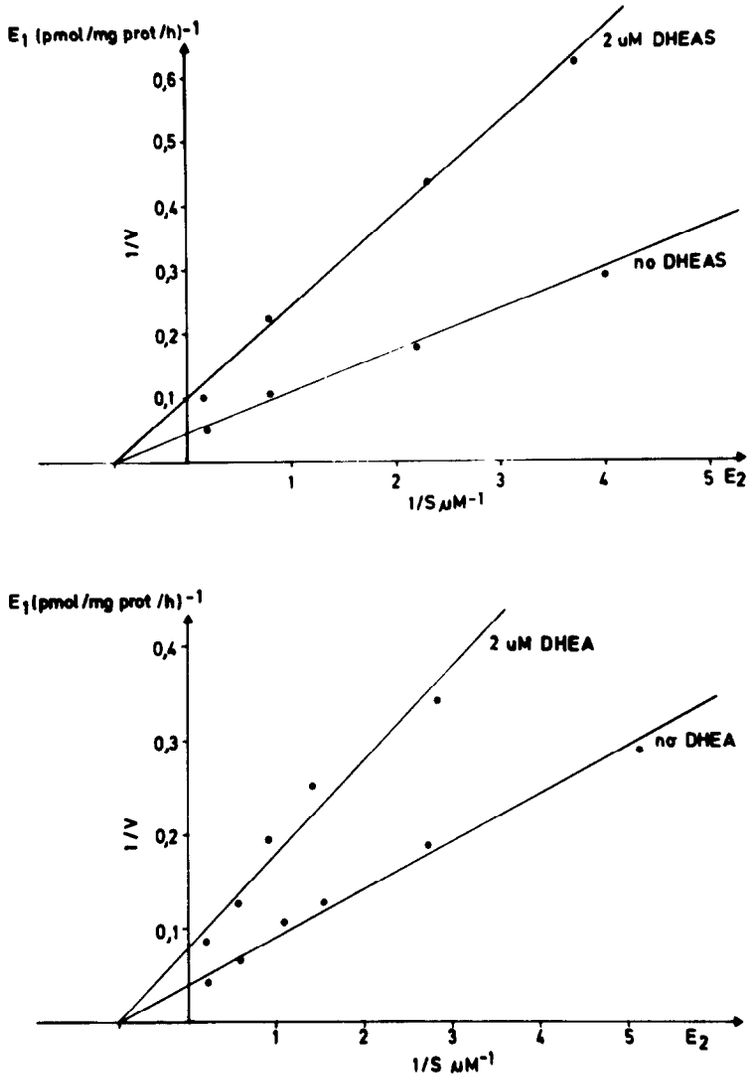

Fig. 3. Non-competitive inhibition of E_2DH activity by exogenous DHEA and DHEAS (2µ.M), respectively.

marker of estrogen action.

Whereas E_1 concentration was higher than E_2 concentrations in plasma, the mean E_2 concentration in either glandular breast or cancer tissue was higher than E_1 concentration (although in 11 out of 50 cancer tissues, E_1 was higher than E_2). Expressed in pg/g tissue, the mean E_2 concentration in both glandular and carcinomatous breast tissue was about 20 times the mean plasma concentration, whereas for E_1 the ratio was about 4, compared to 0.6 for E_1S (Table 1). In normal postmenopausal endometrium, Vermeulen-Meinert *et al.* [44] as well as Wiegerinck *et al.* [45] observed similar tissue/plasma ratios, whereas Van Landeghem *et al.* [36] reported a rather similar gradient in malignant breast tissue from postmenopausal (but not from premenopausal) women. This higher E_2 concentration in the cell is probably related to its binding to the receptor and eventually the presence of non specific binding proteins with low affinity but high capacity [36].

If we consider only receptor positive tissues, the mean E_2 concentration was 0.28 ng/g or $\pm\ 10^{-9}$M. At a mean receptor concentration of 100 fmol/mg protein, or $\pm\ 3 \times 10^{-9}$M and taking a dissociation constant of 10^{9}M [46], this E_2, if freely available, could saturate estradiol receptor sites to 25%, probably sufficient to obtain near maximal biological response [47].

Our data show the presence of an active aromatase in normal glandular and in carcinomatous tissue; the activity in the latter was significantly higher than in the normal tissues.

D'Agata *et al.* [48], failing to find evidence that mammary tumor cell lines aromatize androgens, suggested that aromatization by mammary tumors reflects aromatization by fat cells or normal breast tissue. However, as we observed aromatase activity to be much higher in breast cancer tissue than in either normal parenchymatous tissue or breast fat,

there is little doubt that human breast cancer tissue has an active aromatase. This is in accordance with recent data of several authors [49–55].

In contrast with the data of Abul Hajj [50, 51], who observed aromatase activity (conversion of DHEA and T, respectively, to estrogens) mainly in receptor negative tumors and only in 15% of receptor positive tumors, neither Varela and Dao [49], Li and Adams [56], Tilson-Mallett *et al.* [27] nor ourselves found any correlation between aromatase activity and either estrogen or progesterone receptor status. This strongly suggests that aromatase activity is not a biochemical characteristic of hormone independent tumors. Neither did we observe any correlation between aromatase activity and tissue steroid concentration. Moreover neither age nor BMI appeared to influence aromatase activity, at variance with the increasing aromatization rate with age in human adipose cells reported by Cleland *et al.* [57].

Aromatization of A at V_{max} in breast tissue homogenates under our experimental *in vitro* conditions, yielded ± 20 fmol E_1/mg prot/hr or ± 5 pg/E_1/mg/prot/hr, compared to an estrogen (E_2 + E_1) concentration of ± 20 pg/mg prot. As the concentrations of A and T in breast tissue are at least one order of magnitude lower than the concentration at V_{max}, it would seem that, in as far as *in vitro* activity could be extrapolated to the *in vivo* situation (which is not evident), the contribution of the aromatase to breast tissue estrogen levels is negligible.

Bradlow [58] and Tilson-Mallett *et al.* [27] arrived at a similar conclusion, even taking into account DHEA as a possible precursor. Hence uptake from plasma, as suggested by the work of Duvivier [10], and/or local synthesis from other sources, are probably the major determinants of tissue estrogen concentration.

It has recently been suggested that the major source of estrogens in breast tumors might be estrone sulphate [59, 60], the steroid sulphatase being much more active than the aromatase. Estrone sulphate concentration in plasma in postmenopausal women is indeed much higher than E_1 concentration, but in order to postulate intracellular hydrolysis of E_1S as a major source of intracellular estrogens, one must establish whether it can cross target tissue cell membranes *in vivo*. Whereas for the nonesterified steroids the tissue/plasma concentration gradient is significantly above unity, this is not the case for the sulphate esters and the tissue/plasma gradient of E_1S is below unity. This is possibly related to the polarity of the sulphate, rendering diffusion through the plasma membrane difficult. This is confirmed by the observation of Holinka and Gurpide [61] that, whereas rabbit uterus tissue can convert E_1S to E_1 and E_2, the perfused uterus is unable to do so [61]. E_1S concentration, which is much higher than E_1 concentration in plasma, is comparable to E_1 concentration in breast tissue. Whether this E_1S is taken up from plasma and is transformed to E_1, or whether tissue E_1 undergoes sulphation in the tissue is still unclear, most tumors possessing both the sulphokinase and the sulphatase. Wilking *et al.* [60] observed formation of E_2 from E_1S to be higher in E_2R poor tissue, but as sulphatase activity was unaffected by receptor status, they believe reduction of E_1 to be the rate limiting step. Further studies on the relative activities of sulphokinase and sulphatase will be required to elucidate the eventual role of E_1S as an estrogen precursor in breast cancer tissue.

17β-dehydrogenase activity ($E_2 \rightarrow E_1$) was significantly higher in breast tumor than in normal glandular tissue. This is in accordance with data of Bonney *et al.* [34], but at variance with those of Pollow *et al.* [62]. Furthermore we observed significantly higher ED_2H activity in E_2R + than in E_2R − tumors. This is in contradiction with the data of Abul Hajj *et al.* [63] in mammary tumor and of Tseng *et al.* in endometrium [64], whereas Lubbert and Pollow [15] found no correlation in this respect. Recently Fournier *et al.* [65] reported also a higher E_2DH activity in E_2R + PR + tumors.

Data of Prudhomme *et al.* [67] suggest that E_2DH activity can be considered a good marker for hormone dependency. Our finding of higher E_2DH activity in E_2R positive tumors is in accordance with this view.

We did not observe any influence of age or BMI on E_2DH activity (Table 2). In agreement with the non-competitive *in vitro* inhibition of E_2DH ($E_2 \rightarrow E_1$) by exogenous DHEA(S) in physiological concentration, as reported by Bonney *et al.* [66] in endometrial tissue, we observed a significant negative correlation between DHEA(S) concentration in carcinomatous breast tissues and dehydrogenase activity (Figs. 1 and 2). Tissue DHEA and DHEAS concentrations are however positively correlated ($r = 0.52$, $P < 0.001$), and study of partial correlation reveals that the apparent influence of DHEAS on E_2DH activity can be in fact entirely ascribed to DHEA (partial correlation coefficient − 0.72, $P < 0.001$). Surprisingly, with respect to the *in vitro* inhibition, Bonney *et al.* [34] observed a positive correlation between DHEA(S) concentration and E_2DH activity in breast tumors; the reason for the discrepancy is not evident. As the concentration of exogenous DHEA(S), required to inhibit significantly the E_2DH activity *in vitro* is within the concentration range observed in breast cancer tissue (up to 1.35 μM), the inverse correlation between endogenous DHEA(S) concentration and E_2DH activity reported in this study is not unexpected.

In conclusion, our data suggest that estrogens in mammary cancer tissue originate only to a minor extent from aromatization of androgen precursors; whether uptake of free or of sulphate esters of estrogens could represent a major source requires further studies, but we observed no correlation between plasma and tissue estrogen concentration. The latter is higher in E_2R + than in E_2R − tissues. We found mammary cancer tissue to be characterized by a higher aromatase and steroid E_2DH activity, but lower androgen concentration than normal glandular tissue. Dehydroepiandrosterone and its sulphate, in physiological tissue concentrations, are able to modulate E_2DH activity, inhibiting the conversion of E_2 to E_1. Hence the lower DHEA(S) concentration in cancer tissue in comparison to normal breast tissue could perhaps contribute to the higher E_2DH ($E_2 \rightarrow E_1$) activity, favoring the formation of E_1. In as far as E_2R is a parameter of hormone sensitivity, E_2R + tumors appear to be characterized by higher E_2 and higher E_2DH activity than E_2R −, hormone insensitive tumors. As DHEA(S) inhibits the oxidation of E_2 to the biologically less active E_1, it appears therefore that these androgens might stimulate breast cancer growth. The latter effect of DHEA(S) may constitute a mechanism by which (besides suppression of estrogen precursors) medical and surgical adrenalectomy might exert, at least in part their therapeutic activity in hormone sensitive metastatic mammary cancer. Moreover E_2DH activity might be an additional sensitive marker of hormone dependency of mammary cancers.

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