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Metabolism of Estradiol by Human Breast Cancer*†

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Abstract—*The activities of estrogen-2,4- and 16α-hydroxylase as well as 17β-hydroxysteroid dehydrogenase (17β-OHSDHase) were determined in normal, benign and mammary tumor breast tissues. All three hydroxylases were absent in both normal and benign tumor breast tissues. 17β-OHSDHase was absent from normal breast tissues but present in all benign tumor tissues. Estrogen 2-hydroxylase was present in most breast carcinomas, was significantly higher in estrogen receptor (ER)-positive than ER-negative tumors, did not correlate with progesterone receptor (PR) and was significantly lower in the ER+PR− subgroup of breast cancers. 4-Hydroxylase activity did not correlate with either ER or PR content but was significantly lower in the ER+PR− subgroup of breast cancers. The activity of 16α-hydroxylase was present in only 18% of all tumors investigated (9/50), did not correlate with the ER or PR content and was completely absent in the ER+PR− and ER−PR+ subgroups. The activity of 17β-OHSDHase was significantly higher than the estrogen hyroxylase but did not correlate with either the ER or PR content, and was not different among the ERPR tumor subgroups. The physiological role of these enzymes in the metabolism of estradiol in relation to breast cancer is discussed.*

INTRODUCTION

MALIGNANT mammary cancer tissue has the ability to metabolize steroid hormones. Evidence for this is mainly based on *in vitro* incubation studies, showing that homogenates of breast carcinomas are able to convert androgens to a wide variety of compounds including inactivation products, as well as to other biologically active compounds [1–6]. These findings are further supported by *in vivo* studies in which androgens can be metabolized to a wide variety of conversion products [7, 8]. Of importance is the synthesis of estrogenic compounds by breast cancer, the significance of which is still controversial [9–11]. While considerable work has been carried out on androgen metabolism, little is known about estrogen metabolism by human mammary tumor tissues.

One of the major pathways of estradiol metabolism involves hydroxylation at C-2 or C-4 and C-16 leading to the formation of catechol estrogens and estriol, respectively. These alternate pathways have attracted considerable interest over the past decade, and Martucci and Fishman [12] suggested that the direction of metabolism of estradiol may have proposed that metabolism of estradiol favoring estriol formation prolongs the activity of estradiol while 2-hydroxylation terminates it. More recently, Fishman's group has presented evidence that increased formation of 16α-hydroxyestrone, a metabolite of estradiol, may play a significant role in relation to cirrhosis, systemic lupus erythematosus and breast cancer [13–15].

In view of the fact that little is known about *in vitro* metabolism of estradiol in human breast cancer, we carried out a systematic study to determine the activity of 2,4- and 16α-hydroxylations of estradiol, as well as assessing the activity of 17β-hydroxysteroid dehydrogenase in human mammary tumor tissues. Furthermore, the PR and ER content was determined for all tissues and correlated with enzyme activity.

MATERIALS AND METHODS

Mammary tumor tissues

All tissues were obtained from the Rotterdam

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Radiotherapeutic Institute tumor bank, Rotterdam, The Netherlands, which were kept frozen at −70°C.

Radiolabelled steroids

[2-^{3}H]Estradiol ([2-^{3}H]E_2) and [4-^{3}H]estradiol ([4-^{3}H]E_2) were prepared from 2- and 4-bromoestradiols as described previously [16, 17]. Tritiation was performed by New England Nuclear, and the subsequent purification was carried out in our laboratory. [2-^{3}H]E_2 was found to be 97% pure and [4-^{3}H]E_2 was 98% pure as determined by reverse isotope dilution and bromination of [^{3}H]E_2. [17β-^{3}H]E_2 was obtained by [^{3}H]$NaBH_4$ reduction of estrone as described previously [18]. The synthesis of [16α-^{3}H]E_2 was carried out as described by Fishman *et al.* [19]. Catalytic reduction of Δ^{16}-estradiol-3,17-diacetate was carried out by Amersham to result in formation of [16α,17α-^{3}H]-estradiol-3,17-diacetate that, when treated with $KHCO_3$ in methanolic solution, gave [16α,17α-^{3}H]estradiol. Chromic acid oxidation gave [16α-^{3}H]estrone that, upon reduction with $NaBH_4$, yielded (16α-^{3}H]E_2. The distribution of the tritium at the 16-position was carried out as described by Fishman *et al.* [19] and was found to be 82% 16α- and 18% 16β. [6,7-^{3}H]Estradiol (40–60 Ci/mmole) was purchased from New England Nuclear.

Receptor determination

Receptor sites were measured as described earlier [20]. Aliquots of cytosol were incubated overnight at 4°C with increasing concentrations of [^{3}H]E_2 ($0.3-2 \times 10^{-9}$ M) or [^{3}H]R5020 ($0.3-2 \times 10^{-9}$ M). Values were corrected for non-specific binding by carrying out control incubations containing a 1000-fold molar excess of the anti-estrogen nafoxidine or R5020. Bound and free steroids were separated by dextran-coated charcoal. Results were calculated from Scatchard plot analysis from the binding data corrected for non-specific binding.

Tissue preparation and enzyme assay

(a) *Radiometric procedure.* All tumor tissues were thawed, kept on ice and trimmed to remove all adipose tissue. The tissues were homogenized in 4 volumes of ice-cold 10 mM Tris–HCl buffer (pH 7.4) and centrifuged at 2000 ***g*** for 15 min to remove cell debris. The incubation mixture consisted of tissue homogenate, 3.3 μmole of NADP (or 15 μmole of NAD for 17β-OHSDHase assay); 20 μmole glucose-6-phosphate, 5 units of glucose-6-phosphate dehydrogenase, 25 μmole $MgCl_2$ and 8.3 μmole of [^{3}H]E_2 (0.25 μCi) in a total of 1.0 ml of buffer, and incubated for 1 h at 37°C in a constant shaking bath and terminated by the addition of 2 ml of 1 N HCl and left at 4°C overnight. The precipitated proteins were centrifuged at 3000 ***g*** for 20 min and the radioactivity in the supernatant assayed using Amberlite XAD-2 to separate ^3H_2O from [^{3}H]steroids as described by Numazawa *et al.* [21]. The amount of radioactivity in the ^3H_2O is a direct measure of the enzyme activity. The radiometric technique employed in this study provides a number of advantages, as noted previously [19].

(b) *Product isolation procedure.* To detect radiolabeled metabolic products, incubations were carried out as described above except [6,7-^{3}H]estradiol was used instead of the [2-^{3}H]-, [4-^{3}H]-, [16β-^{3}H]- or [17α-^{3}H]E_2. Following precipitation of protein, the supernatant was extracted three times with 2 volumes of ether. The catechol estrogens, estriol and estrone were purified and isolated as described previously [2, 22, 23]. The purified products were diluted with unlabelled carriers and crystallized to constant specific activity.

Expression and evaluation of results

The enzyme activities are calculated as pmoles of product formed per mg protein per 30 min. Protein concentrations were determined by the biuret test [24].

In the statistical calculations, non-parametric methods were used due to the skewed distribution of the data. The results are reported as the means ± S.E.M. and were analyzed for significance using the Wilcox rank sum test.

RESULTS

The results in Table 1 showing a comparison between the radiometric assay and the product isolation assay for 2-OHase, 4-OHase, 16α-OHase and 17β-OHSDHase clearly indicate that human mammary tumor homogenates are capable of catalyzing the release of radioactivity from specifically ^{3}H-labeled estradiol. The congruency of product formation by both assays for 16α-OHase and 17β-OHSDHase activities validates the radiometric assay. Although the radiometric assay for 2-hydroxylation using rat liver and kidney microsomes was validated previously [21, 25], the results obtained from this study show that for both 2-OHase and 4-OHase activities, the radiometric assay seems to consistently overestimate the levels of 2- and 4-hydroxylation in breast cancer tissues by about 28–35%. While the exact reasons for these differences are not fully understood, it is quite possible that these activities may be due to peroxidatic metabolism as reported previously by Jellinck *et al.* [26] for rat uterine tissues.

Table 2 shows the mean, range and median values for 2-, 4- and 16α-hydroxylases, and 17β-hydroxysteroid dehydrogenase activity in normal, benign and carcinoma breast tissues. All four

Table 1. Comparison of the amount of product using the radiometric assay (^3H_2O) and direct isolation in human mammary tumor homogenates*

Substrate	Tumor	2-OHE_2†	4-OHE_2†	16α-OHE_2†	E_1†
[2-^{3}H]Estradiol	1	632 ± 48	—	—	—
	2	572 ± 36	—	—	—
[4-^{3}H]Estradiol	1	—	299 ± 29	—	—
	2	—	799 ± 43	—	—
[16α-^{3}H]Estradiol	1	—	—	163 ± 18	—
	2	—	—	423 ± 31	—
[17α-^{3}H]Estradiol	1	—	—	—	3310 ± 204
	2	—	—	—	190 ± 18
[6,7-^{3}H]Estradiol*	1	429 ± 33	204 ± 12	148 17	3368 ± 169
	2	411 ± 21	520 ± 34	418 ± 25	202 ± 22

*Products from these incubations were obtained using TLC as described previously [2, 22, 23].
†Values are expressed as pmol/mg protein/30 min of product and presented as the mean ± S.D., $n=3$.

Table 2. Activity of 2-, 4- and 16α-estrogen hydroxylase and 17-OHSDHase in normal and abnormal human breast tissues

Tissue	No. samples	Means ± S.E.M. for enzymes			
		2-OHase	4-OHase	16α-OHase	17β-OHSDH
Normal breast	6	nd*	nd	nd	nd
Benign tumor	7	nd	nd	nd	384 ± 86 (521; 425)
Carcinoma					
ER+PR+	18	334 ± 111 (1941; 222)†	373 ± 125 (1789; 149)	128 ± 61 (850; 0)	1949 ± 1362 (24,838; 266)
ER+PR−	11	134 ± 71 (760; 0)	99 ± 94 (1042; 0)	nd	1907 ± 1434 (16,172; 424)
ER−PR+	4	265 ± 88 (535; 192)	291 ± 71 (270; 297)	nd	271 ± 55 (257; 302)
ER−PR−	17	330 ± 117 (959; 265)	340 ± 109 (867; 97)	67 ± 38 (626; 0)	3359 ± 2007 (30,376; 339)
ER+	29	258 ± 76 (1941; 99)	269 ± 88 (1789; 0)	80 ± 39 (850; 0)	1933 ± 989 (24,895; 290)
ER−	21	317 ± 63 (959; 239)	195 ± 45 (867; 164)	55 ± 31 (626; 0)	2770 ± 1637 (30,367; 308)
PR+	22	322 ± 93 (1941; 222)	360 ± 103 (1789; 198)	105 ± 51 (850; 0)	1644 ± 1118 (24,838; 266)
PR−	28	253 ± 55 (959; 122)	142 ± 48 (1042; 41)	41 ± 23 (626; 0)	2789 ± 1329 (30,414; 367)

Results are expressed as pmol/mg protein/30 min of product formed. See details under Materials and Methods section.
*nd = not detectable.
†Values in parentheses represent range and median, respectively.

enzyme activities were carried out for each tissue investigated. No hydroxylase activity was detected in either normal or benign breast tumor tissues. On the other hand, 17β-OHSDHase activity was detected in both benign and carcinoma breast tumors but not in normal breast tissues. For both 2- and 4-hydroxylase activities, statistically significant differences were obtained between carcinoma and benign ($P < 0.0001$) and between carcinoma and normal tissues ($P < 0.0001$). However, no significant differences were obtained for 16α-OHase activity between the carcinoma tissues and either benign or normal breast tissues. It is worth noting that even though the mean and median values for

Table 3. Estrogen-metabolizing enzymes in ER+PR+ human breast carcinoma

Tumor No.	2-OHase	4-OHase	16α-OHase	17β-OHSDHase
1	1941	1534	590	247
2	nd	229	nd	2853
3	636	193	nd	104
4	240	483	nd	320
5	nd	712	nd	1684
6	204	nd	nd	284
7	906	nd	nd	2035
8	81	nd	nd	89
9	353	1789	nd	889
10	342	97	nd	113
11	112	480	nd	290
12	nd	nd	nd	604
13	283	203	nd	136
14	572	799	423	190
15	99	94	437	57
16	nd	nd	nd	24,895
17	nd	nd	850	81
18	253	105	nd	220

Results are expressed as pmol/mg protein/30 min of product formed. See details under Materials and Methods section.

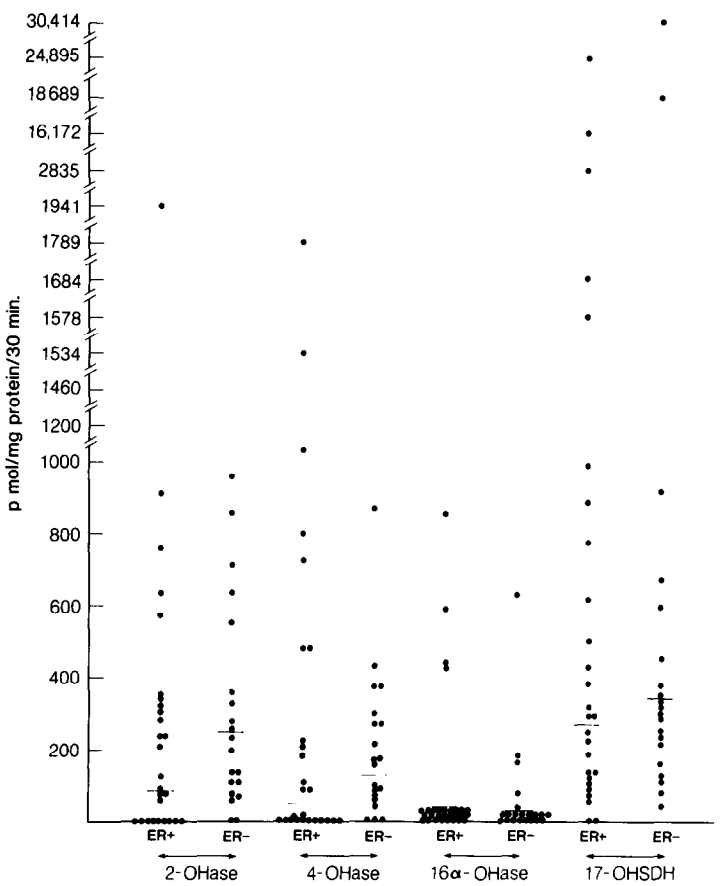

Fig. 1. Relationship between 2-, 4- and 16α-hydroxylase activity and 17β-hydroxysteroid dehydrogenase activity with the estrogen receptor content in human mammary tumors. (Horizontal bars represent median.)

2- and 4-hydroxylase activities are not significantly different, the results shown in Table 3 for the ER+PR+ group indicate that there is significant variation in enzyme activities within each tissue investigated. Essentially similar results were observed for the other ERPR subgroups (data not presented).

Fifty malignant tumors were analyzed for a possible correlation between enzyme activity and ER and PR binding capacity. The results are summa-

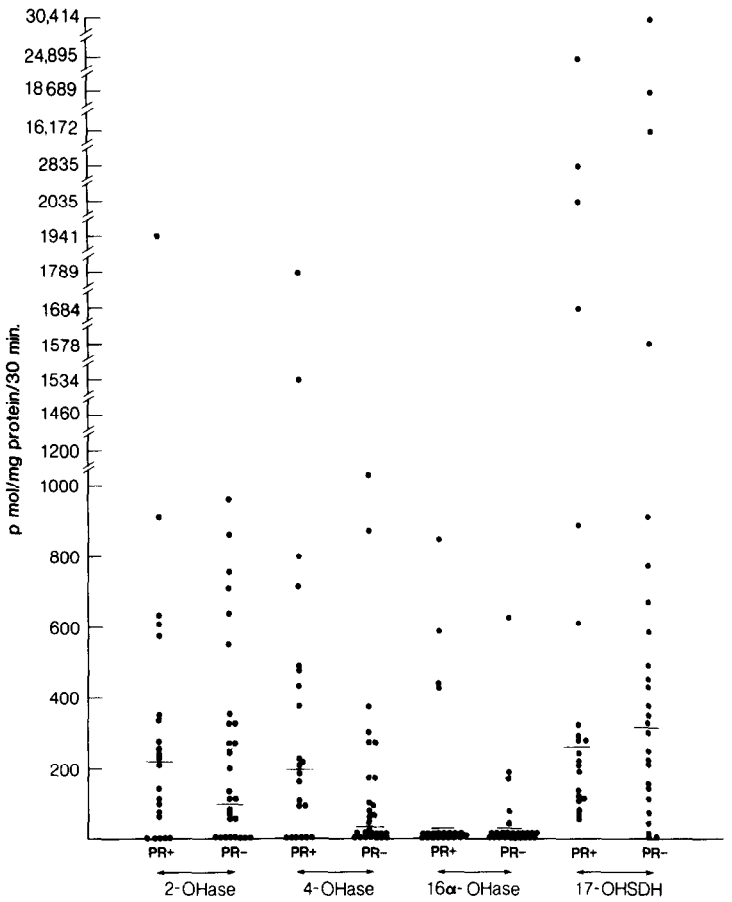

Fig. 2. Relationship between 2-, 4- and 16α-hydroxylase activity and 17β-hydroxysteroid dehydrogenase activity with the progesterone receptor content in human mammary tumors. (Horizontal bars represent median.)

rized in Figs. 1, 2 and 3. As seen from Figs. 1 and 2, there was no correlation between all the enzyme activities and the presence or absence of either ER or PR except for 2-OHase activity in ER+ vs. ER− tumors ($P < 0.05$). Analysis of data presented in Fig. 3 shows that the activity for 2-OHase in the ER+PR− group was significantly lower than the ER+PR+ ($P < 0.0001$), ER−PR+ ($P < 0.001$) and ER−PR− ($P < 0.0001$). Similarly, the activity for 4-OHase in the ER+PR− group was found to be significantly lower than the ER+PR+ ($P < 0.0001$), ER−PR+ ($P < 0.0001$) and ER−PR− ($P < 0.004$). The activity for 17β-OHSDHase was not significantly different in all receptor groups investigated, while the 16α-OHase activity was detected in only a small number of ER+PR+ and ER−PR− subgroups.

DISCUSSION

The experiments described in this report show that human mammary tumors are capable of catalyzing the metabolism of estradiol to the catechol estrogens (2-OH-E_2 and 4-OH-E_2) as well as to estrone, indicating the presence of 2-OHase, 4-OHase and 17β-OHSDHase in mammary tumors. These results are essentially similar to those reported earlier by several groups [27–29]. However, in our studies we could not detect the presence of 2-OHase in benign tumors as reported by Hoffman *et al.* [28].

The activity of 17β-OHSDHase did not correlate with either the presence or absence of ER or PR. These results are slightly different from those reported earlier by us [27] in which the activity of 17β-OHSDHase was found to be higher in the ER− tumors than in the ER+ tumors. The levels of 2-OHase and 4-OHase activities did not correlate with either the ER or PR levels in the tissues investigated. These results are similar to those reported by Purdy and Wittliff [29]. Furthermore, these workers showed that the relative ratios of the rates of 2-/4-hydroxylase activity increased from a mean of 5.7 in the ER− group to 19.6 in the ER+ group. Our studies do not seem to show similar results. It is interesting to note that the relative activities of 2- and 4-hydroxylases in the ER+PR− tumors (Fig. 3) are significantly lower than that observed in the other three receptor subgroups. Although the ER+PR+ breast carcinoma tissues are highly differentiated and are close to the normal

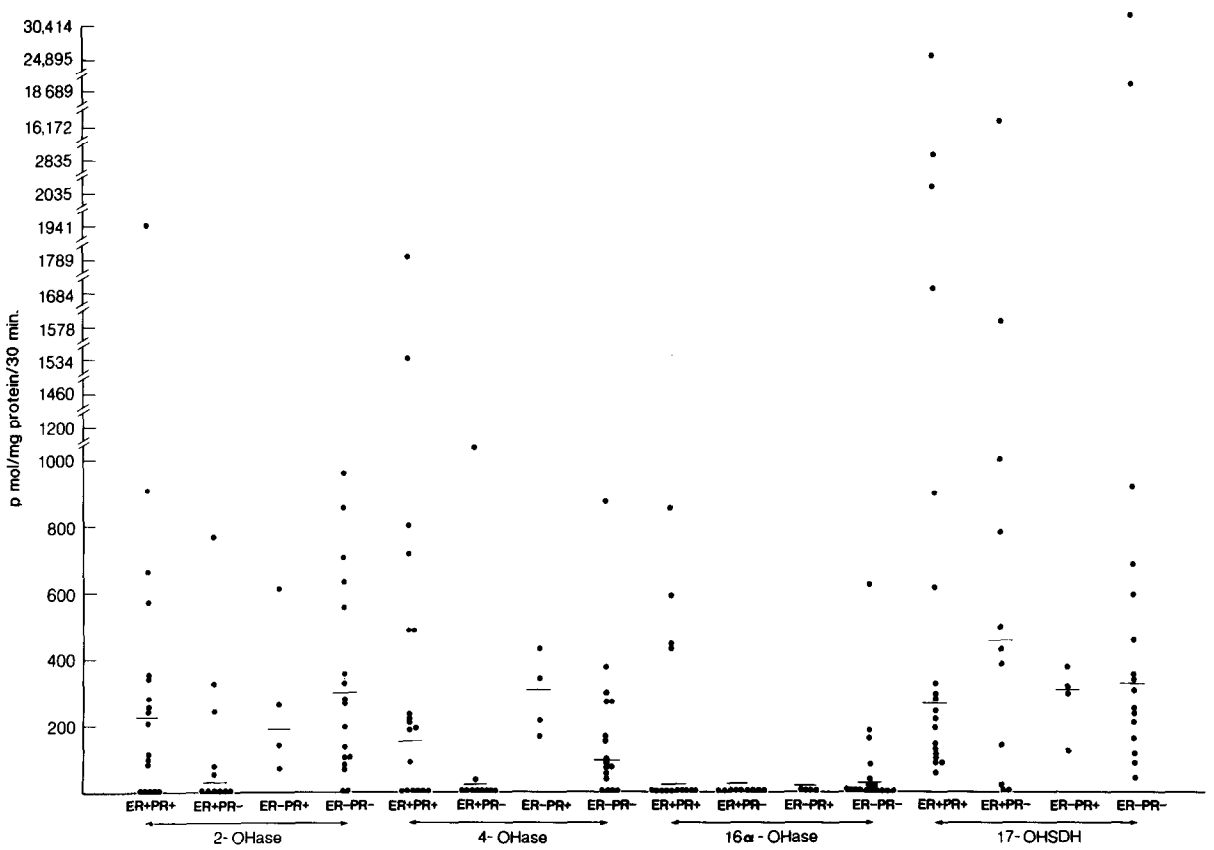

Fig. 3. Relationship between 2-,4- and 16α-hydroxylase activity and 17β-hydroxysteroid dehydrogenase activity with the estrogen and progesterone receptor contents in human mammary tumors. (Horizontal bars represent median.)

breast structure, these tumors showed appreciable 2- and 4-OHase, and 17β-OHSDHase activities which are not detectable in the normal breast tissues. It does seem that transformation of cells from a normal structure to a highly differentiated tumorous structure leads to significant changes in enzyme activity. The results obtained from these studies are not unique and are essentially similar to those obtained for ER status and aromatase activity (data not presented) in highly differentiated breast carcinoma.

One of the most significant observations in this study is the fact tht most mammary tumors lack 16α-OHase activity. This is essentially similar to our earlier studies using human mammary tumors [1] but is different from those reported in the GR-mouse mammary tumor systems [23] in which appreciable amounts of 16α-OHase was detected. Earlier studies by several groups demonstrated the presence of 16α-hydroxylase in mammary tumors [3, 30]; however, the identity of the products was not unequivocally established. The results reported in this study show that only 18% of the tumor tissues showed 16α OHase activity.

The recent findings by Schneider *et al.* [14] that women with breast cancer show increased 16α-hydroxylation cannot be explained by increased 16α-OHase activity in mammary tumor tissues as shown in this study. The question as to what tissue(s) may be responsible for the increased 16α-hydroxylation in women with breast cancer has yet to be determined. 16α-Hydroxylation of estrogens has been shown to take place primarily in liver [31, 32]. Thus, it is quite possible that the increased 16α-hydroxylation observed by Schneider *et al.* [14] in breast cancer patients may be due to increased stimulation of 16α-hydroxylase in the host livers of these patients. However, the evidence from rat model studies does not support this conclusion. Studies carried out in our laboratories [33] on the effect of Morris hepatomas on the 16α-hydroxylase activity in host livers shows a slight but significant decrease in 16α-hydroxylase activity in the liver of tumor-bearing rats. It would be quite interesting to determine the tissue site in which 16α-hydroxylase activity is increased in women with breast cancer.

The question that needs to be answered is: what is the physiological significance of the presence of the estrogen 2- and 4-hydroxylases and the 17β-hydroxysteroid dehydrogenase in breast tumor tissues? It is well established that metabolism of estradiol in its target tissues provides a mechanism for changing the potency and diversifying the actions of the parent hormone at specific sites. Thus, 17β-OHSDHase converts estradiol to estrone which is substantially less active, 2-hydoxylation of

estradiol may result in a reduction in estrogenic potency of the molecule [34, 35] or even antagonistic properties [36], and 4-hydroxylation does not appear to reduce the estrogenic potency of estradiol [36]. While it is quite possible that breast tumors might act to metabolize estradiol leading to a more favorable environment for tumor growth, the results obtained from this investigation clearly show a marked increase in 2- and 4-OHases and 17β-OHSDHase in breast carcinoma tissue with very little or no 16α-OHase activity. Whether the direction of metabolism of estradiol leading to the increased formation of the catechol estrogens and estrone and less toward 16α-hydroxylation in breast tumors is in any way related to tumor growth and maintenance remains to be elucidated.

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REFERENCES

1. Abul-Hajj YJ. Metabolism of dehydroepiandrosterone by hormone dependent and hormone independent human breast carcinoma. *Steroids* 1975, **26**, 488–500.
2. Abul-Hajj YJ, Iverson R, Kiang DT. Aromatization of androgens by human breast cancer. *Steroids* 1979, **33**, 205–222.
3. Adams JB, Wong MSF. Paraendocrine behavior of human breast carcinoma: *in vitro* transformation of steroids to physiologically active hormones. *J Endocrinol* 1968, **41**, 41–52.
4. Griffiths K, Jones D, Cameron EHD, Gleave EN, Forrest APM. Transformation of steroids by mammary cancer tissue. In: Dao TL, ed. *Estrogen Target Tissues and Neoplasia*. Chicago, University of Chicago Press, 1972, 151–162.
5. Miller WR, Forrest APM. Oestradiol synthesis by a human breast carcinoma. *Lancet* 1974, , 866–868.
6. Varela R, Dao TL. Estrogen synthesis and estradiol binding by human mammary tumors. *Cancer Res* 1978, **38**, 2429–2433.
7. Deshpande N, Carson P, DiMartino L, Tarquini A. Biogenesis of steroid hormones by human mammary gland *in vivo* and *in vitro*. *Eur J Cancer* 1976, **12**, 271–276.
8. Van Landeghiem AAJ, Poortman J, Deshpande N *et al.* Plasma concentration gradient of steroid hormones across human mammary tumors *in vivo*. *J Steroid Biochem* 1981, **14**, 741–747.
9. Abul-Hajj YJ. Comparative studies of aromatase inhibitors in relation to the significance of estrogen synthesis in human mammary tumors. *Cancer Res* 1982, **42**, 3373s–3377s.
10. Bradlow HL. A reassessment of the role of breast tumor aromatization. *Cancer Res* 1982, **42**, 3383s–3386s.
11. Miller WR, Hawkins RA, Forrest APM. Significance of aromatase activity in human breast cancer. *Cancer Res* 1982, **42**, 3365s–3368s.
12. Martucci C, Fishman J. Direction of estradiol metabolism as a control of its hormonal action–uterotropic activity of estradiol metabolites. *Endocrinology* 1977, **101**, 1709–1715.
13. Lahita R, Bradlow HL, Kunkel HG, Fishman J. Alterations of estrogen metabolism in systemic lupus erythematosus. *Arthritis Rheum* 1979, **22**, 1195–1198.
14. Schneider J, Kinne D, Fracchia A *et al.* Abnormal oxidative metabolism of estradiol in women with breast cancer. *Proc Natl Acad Sci USA* 1982, **79**, 3047–3051.
15. Zumoff B, Fishman J, Gallagher TF, Hellman L. Estradiol metabolism in cirrhosis. *J Clin Invest* 1968, **47**, 20–26.
16. Fishman J, Guzik H, Hellman L. Aromatic ring hydroxylation of estradiol in man. *Biochemistry* 1970, **9**, 1593–1598.
17. Williams JG, Longcope C, Williams KIH. 4-Hydroxyestrone: a new metabolite of estradiol-17β from humans. *Steroids* 1974, **24**, 687–701.
18. Fishman J, Bradlow HL, Zumoff B, Hellman L, Gallagher TF. Further studies of the metabolism of estradiol in man. *Acta Endocrinol* 1961, **37**, 57–62.
19. Fishman J, Bradlow HL, Schneider J, Anderson KE, Kappas A. Radiometric analyses of biological oxidations in man: sex differences in estradiol metabolism. *Proc Natl Acad Sci USA* 1980, **77**, 4957–4960.
20. Poortman J, Thijssen JJH, Schwarz F. Steroid receptors in human reproductive tissues. In: Wittliff JL, Dapunt O, eds. *Steroid Receptors and Hormone Dependent Neoplasia*. New York, Masson, 1980, 45–58.
21. Numazawa M, Kiyono Y, Nambara T. A simple radiometric assay for estradiol 2-hydroxylase activity. *Anal Biochem* 1980, **104**, 290–295.
22. Hersey RM, Williams KI, Weisz J. Catechol estrogen formation by brain tissue: characterization of a direct product isolation assay for estrogen 2- and 4-hydroxylase activity and its application to studies of 2- and 4-hydroxy estradiol by rabbit hypothalamus. *Endocrinology* 1981, **109**, 1912–1920.
23. Abul-Hajj YJ, Kiang DT. Metabolism of testosterone by GR mouse mammary tumors. *Cancer Res* 1982, **42**, 3510–3513.
24. Layne E. Spectrophotometric and turbidometric methods for measuring proteins. *Methods*

Ezymol 1957, **3**, 450–454.
25. Kupfer D, Miranda GK, Bulger WH. A facile assay for 2-hydroxylation of estradiol by liver microsomes. *Anal Biochem* 1980, **116**, 27–34.
26. Jellinck PH, Norton B, Fishman J. Formation of ^3H_2O from [2-^{3}H]- and [4-^{3}H]estradiol by rat uteri *in vitro*: possible role of peroxidase. *Steroids* 1980, **35**, 579–589.
27. Abul-Hajj YJ, Iverson R, Kiang DT. Estradiol 17β-dehydrogenase and estradiol binding in human mammary tumors. *Steroids* 1979, *33*, 477–484.
28. Hoffman AR, Paul SM, Axelrod J. Catechol estrogen synthesis and metabolism by human mammary tumors *in vitro*. *Cancer Res* 1979, **39**, 4584–4587.
29. Purdy RH, Wittliff JL. Rates of metabolic activation of estradiol versus estrogen (ER) and progesterone receptor (PR) levels in human breast cancer. *Proc Am Assoc Cancer Res* 1984, **25**, 217.
30. Dao TL, Varela R, Morreal CE. Metabolic transformation of steroids by human breast cancer. In: Dao TL, ed. *Estrogen Target Tissue and Neoplasia*. Chicago, University of Chicago Press, 1972, 163–179.
31. Brener H, Knuppen R. Enzymes of estrogen metabolism. *Methods Enzymol* 1969, **15**, 691–735.
32. Brodie BB, Gillette JR, LaDu BN. Enzymatic metabolism of drugs and other foreign compounds. *Ann Rev Biochem* 1958, **27**, 427–452.
33. Abul-Hajj YJ, Morris HP. Metabolism of estrogens in hepatomas of different growth rates. *Cancer Res* 1977, **37**, 1083–1087.
34. Ball P, Knuppen R. Catecholestrogens (2- and 4-hydroxyestrogens): chemistry, biosynthesis, metabolism, occurrence and physiological significance. *Acta Endocrinol* 1980, **93**, 1–127.
35. Hersey R, Weisz J, Katzenellenbogen BS. Estrogenic potency, receptor interactions and metabolism of catechol estrogens in the immature rat uterus *in vitro*. *Endocrinology* 1982, **111**, 896–903.
36. Schneider J, Huh MM, Bradlow HL, Fishman J. Antiestrogen action of 2-hydroxyestrone on MCF-7 human breast cancer cells. *J Biol Chem* 1984, **259**, 4840–4845.