Journal of Steroid Biochemistry, 1976, Vol. 7, pp. 897–903. Pergamon Press. Printed in Great Britain

STEROID ABNORMALITIES IN ENDOMETRIAL AND BREAST CARCINOMA: A UNIFYING HYPOTHESIS*

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SUMMARY

Studies were carried out to further identify the nonendocrine tissues in which peripheral estrogen synthesis takes place. To test the hypothesis that adipose tissue is an important site the conversion of androstenedione (A) to estrone (E1), $[\rho]^{A\text{-}E1}$, was measured in four obese women before and after enforced weight reduction (mean weight loss, 89 lbs). Unexpectedly the $[\rho]^{A\text{-}E1}$ value increased in each subject following weight loss. These results suggest that the relationship between obesity and liver abnormalities may be more important than obesity alone in determining $[\rho]^{A\text{-}E1}$. A variety of human tissues were also assayed for their ability to form E1 from A and progesterone from pregnenolone. Normal breast and other tissues had low or undetectable activities and only 1 of 4 breast tumors had significant aromatase activity. Both normal and tumorous colon and rectum had relatively high progesterone forming activity whereas other tissues were inactive. These results are discussed in relation to previously reported abnormalities in steroid production and metabolism observed in breast and endometrial cancer patients.

INTRODUCTION

Extensive studies in recent years have demonstrated that the major if not exclusive source of estrogen in anovulatory women is peripheral aromatization of circulating androstenedione to estrone [1 - 4]. This mechanism is operative in postmenopausal women as well as in young subjects in whom ovulatory function has been disrupted as in polycystic ovarian disease and ovarian tumors. Furthermore, it has been established that this mechanism of estrogen production is subject to metabolic influences such as the presence of obesity and liver disease in which excessive estrone production is frequently encountered. Of particular interest has been the correlation between excessive extraglandular estrone production, either by increased efficiency for conversion or increased availability of precursor androstenedione, with an increased risk of endometrial carcinoma. Thus a unified concept had emerged which provides a firm link between the diverse constitutional features and ovarian alterations that are frequently found in endometrial carcinoma patients and excessive production of estrone rather than the ovarian estrogen, estradiol. These observations form the basis of a working hypothesis concerning the role of estrogens in this and other neoplasms of estrogen target tissues which provides new insight and potential for understanding both the endocrine abnormalities associated with these diseases and the beneficial effects of endocrine therapy [5,6].

While the basic mechanism of peripheral estrogen production in the human has been defined, the nonendocrine tissue sites of estrogen synthesis have not been well characterized. Only a few isolated studies of human adult tissues for their capacity to produce estrogen have appeared. Early studies showed that the most likely candidate, liver, was extremely inefficient in this regard, with negative findings being reported by most investigators [7]. It should be noted that this is in marked contrast to fetal liver in which aromatization is easily demonstrated. More recently, the capacity of adipose tissue to aromatize androstenedione, albeit in very low yields, has been reported [8,9]. These findings support the concept of estrogen production by adipose tissue suggested by the highly significant positive correlation found between body weight and extent of conversion of androstenedione to estrone observed in both men and woman [4].

However, alternative explanations may be put forth for increased peripheral estrogen production, since changes in production and metabolism of adrenal steroids are known to occur in obesity. Enhanced availability of adrenal androstenedione to aromatase enzyme sites could result from an increased secretion rate or decreased hepatic clearance via normal metabolic pathways, i.e. conversion to 17-ketosteroids. Alternatively the significant degree of fatty metamorphosis without clinically detectable changes in liver function commonly found in obesity [10] may be associated with enhanced estrogen

*Supported in part by American Cancer Society Grant BC33 and NIH Grant 8692.
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synthesis within the liver itself. In order to explore these possibilities we have measured the peripheral conversion of androstenedione to estrone in a group of obese women before and after enforced weight reduction. In addition, we have surveyed a variety of normal and tumorous human tissues for their ability to form estrone from androstenedione and also to form progesterone from pregnenolone. The latter study was prompted by our recent finding that progesterone in high local concentrations exerts remarkable anti-inflammatory and/or immunosuppressive activity [11].

MATERIALS AND METHODS

The *in vivo* extent of conversion of androstenedione $[\rho]^{A\text{-}E1}_{E\text{-}U}$, was measured by the urinary method as previously described [3]. Forced weight reduction in two obese women was accomplished by ileojejunal bypass surgery and in two others by maxillary-mandibular fixation. A complete description of the clinical and other aspects of this study will be published elsewhere [12]. Screening assays for aromatase activity were performed using the ^3H_2O release assay [13] in slightly modified form. In the studies to be reported, estrone and estradiol formation from adrostenedione was assayed by chromatographic purification and crystallization to constant isotope ratios as described previously [14]. Conversion of pregnenolone to progesterone was assayed as follows: [7-^{3}H]-pregnenolone (1nM) in Krebs - Ringer buffer was incubated with 50 mg of tissue for 1 h at 37°C. The incubation mixture was extracted with chloroform, authentic ^{14}C tracers added, and progesterone was isolated by liquid - liquid chromatography, silica gel thin layer chromatography, and crystallization of products, as previously described [14].

RESULTS AND DISCUSSION

Shown in Table 1 are the results of measurements of the per cent conversion of androstenedione to estrone ($[\rho]^{A\text{-}E1}$) before and after an average weight loss of 89 lbs in 4 women. Surprisingly, the $[\rho]^{A\text{-}E1}$ value increased in each subject, the average value increasing from 2.4% (1.3 - 4.7) to 3.2% (2.5 - 4.8). Consistent with these observations was the fact that the plasma levels of estrone increased in 3 of the 4 subjects (data not shown). Assessment of liver function in these subjects showed no gross abnormalities before or after weight reduction. While more extensive studies are needed, it is evident from the above studies that the extent of conversion of androstenedione to estrone is not determined simply by the degree of obesity. Although liver function tests showed no consistent changes resulting from weight loss in these subjects, histologic evidence of hepatic fatty degeneration following starvation has been reported [15]. Also similar changes are frequently encountered in obese subjects [10]. Thus it is possible that the effects of starvation superimposed upon the fatty changes found in obese subjects may increase estrogen production even in the absence of clinically evident liver malfunction. It is important to note that severe liver disease, as in alcoholic cirrhosis, is associated with markedly increased peripheral estrogen synthesis [4] and elevated plasma estrogen levels [16].

The physiologic mechanisms that give rise to increased estrogen synthesis in patients with liver abnormalities are obscure. If indeed adipose tissue is the major tissue site of the aromatase enzyme then increased conversion of androstenedione to estrone following weight loss could be explained by a reduction in the hepatic clearance of adrostenedione to nonaromatizable steroids such as the 17-ketosteroids. Thus increased availability of precursor androstenedione to an unchanged quantity of aromatase enzyme in adipose cells would explain the increased values of $[\rho]^{A\text{-}E1}$. This explanation would be consistent with observations indicating that the degree of obesity is determined by lipid content of adipose cells and not their total number. However, this mechanism implies that major changes in hepatic clearance of androstenedione occur in severe hepatic disease since conversion of androstenedione to estrone may be elevated as much as 5-fold [4]. In this event a decrease in the total metabolic clearance rate of androstenedione (MCR_A) would be expected since splanchnic extraction accounts for more than half of the total MCR_A [17]. Indeed, the MCR of testosterone was reduced about 40% in men with cirrhosis whereas the peripheral conversion to estradiol $[\ ]^{T\text{-}E2}$ was increased from 0.32 to 0.59% [16]. While these findings suggest that increased

Table 1. Conversion of androstenedione to estrone before and after weight reduction

		Weight			$\Delta^4 \rightarrow$ E1 (%)	
Subject	Age	Before	After	Loss	Before	After
P.F.*	51	277	215	62	2.0	2.9
W.S.*	33	291	199	92	4.7	4.8
D.D.**	18	272	204	68	1.3	2.5
D.P.**	33	349	215	134	1.5	2.4
Mean	33.8	297	208	89	2.4	3.2

*Maxillary mandibular fixation
**Ileojejunal bypass

extrahepatic aromatization, other factors such as alterations in blood flow in fat depots may also be important during weight reduction. It is also possible that adipose tissue is not the only site of transformation but rather, estrogen synthesis from androstenedione may occur in other tissues including the liver itself. Although presently available evidence suggests that normal adult liver does not contain the aromatase enzyme, no data is available for abnormal liver. Thus it is possible that the changes in liver morphology associated with obesity or weight reduction may stimulate estrogen synthesis by hepatic cells. This could result from alterations in the normal distribution of androstenedione amongst the various steriod metabolizing enzymes or from enhanced levels of the aromatase enzyme. The latter could occur if the regenerating liver has some of the characteristics of fetal liver. Experiments are in progress to test this latter possibility. It is clear that much work remains to be done before the mechanisms controlling peripheral estrogen synthesis are unraveled. Indeed, the function, if any, of estrogen produced in nonendocrine tissues is completely unknown.

Some years ago, Wong and Adams[18] and Dao *et al.* [19] independently reported the capacity of breast cancers to produce estrogens from a variety of substrates and suggested a paraendocrine function for such tumors. Several recent reports appear to confirm the fact that at least some human breast tumors contain the aromatase enzyme [20,21]. Our results also suggest that a limited number of tumors may acquire the ability to synthesize estrogens. It is tempting to speculate that the acquisition by tumors of trophoblastic function, i.e. production of placental proteins such as HCG [22] and biologically active steroids may be of importance in tumor biology. For example, small amounts of estrogen arising in the tumor (or adjacent fatty tissue) could be effective in promoting breast tumor growth but go undetected in blood or urine. The finding that certain tumors can form progesterone from pregnenolone, another steroidogenic characteristic of trophoblastic cells, may also be of importance in tumor biology. Our recent studies suggest that progesterone in high local concentrations may suppress certain functions of cell mediated immunity

Table 2. Steroid formation by human tumors

		pM/h/g	
Number	Tumor	Estrone	Progesterone
1.	Breast (metastatic)	1.0 (0)*	0
2.	Breast (fibroadenoma)	0 (0)	—
3.	Breast (fibroadenoma)	0 (0)	—
4.	Breast (adenocarcinoma)	35.5	—
5.	Breast (gynecomastia)	0	—
6.	Breast (cytosarcoma)	0	—
7.	Colon (adenocarcinoma)	1.0 (0)	14.7 (14.7)*
8.	Colon (metastatic)	0.5 (0)	8.4 (4.7)
9.	Colon (metastatic)	0.3 (0)	26.6 (6.4)
10.	Colon (adenocarcinoma)	0 (0)	7.3 (0.2)
11.	Colon (adenocarcinoma)	0 (0)	7.2 (5.3)
12.	Gastric (adenocarcinoma)	0 (0)	0 (0)
13.	Renal (carcinoma)	0.3 (0)	0 (0)
14.	Rectal (adenocarcinoma)	2.1 (1.0)	191 (67)
15.	Rectal (adenocarcinoma)	0.5 (0.4)	139 (38)

*Numbers in ( ) indicate results from corresponding normal tissues.

Table 2 presents the results of *in vitro* assays of a variety of human normal tissues and tumors for their ability to form estrone from androstenedione and progesterone from pregnenolone. It can be seen that very few of the normal or tumor samples had significant aromatase activity. Interestingly, one breast tumor specimen had a much bigger value than any other tissue assayed in these experiments. Also of interest is the fact that both normal and tumorous samples of the rectum and 3 or 5 tumors of the colon possessed measurable activity. This is the first demonstration of aromatase activity in tumors of organs other than the breast. Interestingly, all of the normal tissue and tumor samples of the colon and rectum also demonstrated measurable activity in forming progesterone from pregnenolone. In all but one instance the tumor activity was considerably higher than that of the corresponding normal tissue. [11]. It is of interest that the normal tissue adjacent to those colon tumors which had high progesterone producing capacity also were much higher than other normal tissues. These results indicate that the level 3ß-hydroxy steroid dehydrogenase enzyme varies considerably amongst tissues and that this enzyme may play a role in determining the hormonal milieu of nonendocrine tissues. However, the results from these *in vitro* studies must be interpreted with great caution particularly since the observed activities are very low.

Our previous studies have demonstrated that the anti-breast cancer compound, Teslac, which has been considered to be a very weak androgen is an effective inhibitor of estrogen synthesis both *in vitro* and *in vivo* [23]. More recently, we have shown that Teslac also inhibits the conversion of androstenedione to estrone in women with breast cancer [24]. Thus a

new conceptual basis for therapy of breast cancer and a variety of other abnormalities arising from hyperestrogenism has been established. Other drugs undergoing clinical trials for breast cancer, such as aminoglutethimide (AG), likely produce the same result [25]. The rationale for use of AG has been to produce chemical adrenalectomy since this compound blocks steroid synthesis at an early step. However, we showed several years ago that AG is a very potent inhibitor of the aromatase enzyme [26,13]. Theoretically speaking, therefore, AG is an ideal drug since it reduces androgen production and specifically blocks estrogen synthesis.

That peripheral estrogen synthesis can be reduced pharmacologically raises the question of endogenous factors which may inhibit this system. We have proposed that the 5α-reduced C_{19} steroids may be important since 5α-androgens are effective competitive inhibitors of the aromatase enzyme whereas 5ß-androgens are inactive [23]. Several years ago Bulbrook found that the 5α/5ß ratio of urinary 17-ketosteroids (androsterone/etiocholanolone) was significantly different in women of high (British) and low (Japanese) incidence breast cancer areas [27]. The 5α/5ß ratio was similar in both groups until about age 50 when the ratio in Japanese women increased markedly. The change coincides with the age at which the breast cancer incidence diverges, i.e. there is no significant difference in cancer incidence before 45 - 50. Interestingly, the relative activities of 5α and 5ß reductases appear to be controlled by thyroid hormone [28]. 5α-Reductase activity is increased in hyperthyroid patients or by administration of thyroxine and is decreased in hypothyroid subjects. Thus, thyroid hormone may indirectly regulate peripheral estrogen production by increasing C_{19} steroid metabolism to 5α-reduced compounds which cannot be aromatized and which also inhibit the aromatase enzyme. This may be relevant to a number of early studies (summarized by Hayward, [29]) that suggested an association between hypothyroidism and increased risk of breast cancer. Indeed, some authors have reported that treatment with thyroid extracts reduced the incidence of recurrence of breast cancer. In another study of 196 patients treated with thyroid hormones for 12 years following thyroidectomy, *no* breast cancer was found. In searching for other abnormalities in androgen metabolism Bulbrook studies adrenal dehydroisoandrosterone sulfate (DS) production rates in breast cancer patients and found them to be markedly lower than those in carefully selected controls [30]. Since DS is the major precursor of both androsterone and etiocholanolone [31], low DS production is associated with reduced urinary and plasma levels of the 5α steroid androsterone. In this situation increased tissue conversion of androstenedione to estrone could occur. All of these diverse endocrine abnormalities observed in breast cancer patients could, theoretically at least, increase peripheral estrogen production.

Taken together with the abnormalities of estrogen production associated with obesity and liver disease, these considerations suggest that peripheral estrone

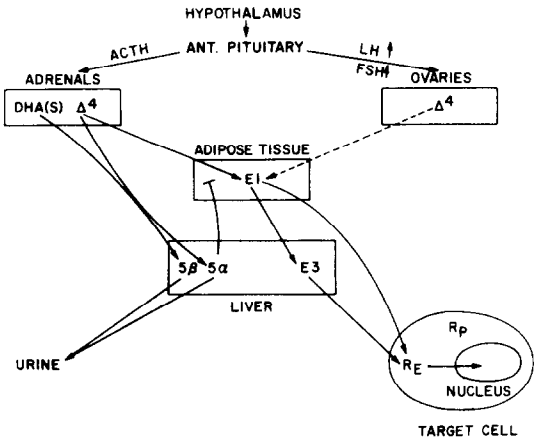

Fig. 1. Schematic illustration of androgen - estrogen relationships in posmenopausal and young anovulatory women. Androstenedione (Δ^4) secreted by the adrenal glands (and ovaries in young anovulatory women) is converted to estrone in adipose tissue and is the major estrogen available to target tissues. Dehydroisoandrosterone sulfate, DHA(S), is also secreted by the adrenals and in converted to 5α and 5ß-reduced steroids in the liver. It is postulated that the level of 5α steroid production may play a regulatory role in estrone production by inhibition of the aromatase enzyme. Estrone interacts with target cell estrogen receptor, R_E, to promote cellular proliferation whereas progesterone is not available to interact with its receptor R_P. Not shown is the conversion of Δ^4 and DHAS to testosterone which is known to occur. See text for additional details.

production from androstenedione may play a central role in development of both breast and endometrial cancer as shown in Fig. 1. This scheme is applicable to both postmenopausal and younger women who are anovulatory for any reason. Anovulatory women, particularly those with polycystic ovaries or ovarian tumors, produce ovarian androstenedione in addition to that derived from the adrenal glands. For example, young women with polycystic ovarian disease produce 3 - 4 times higher than normal amounts of androstenedione which gives rise to high estrone levels despite a normal efficiency of conversion [4]. The higher incidence of endometrial cancer in such women is well documented [32]. Grattarola has reported that the majority of breast cancer patients in his series had long periods of anovulation [33,34], and Korenman more recently also commented upon the frequency of menstrual abnormalities in breast cancer patients [35]. Thus production of estrone in peripheral tissues for long periods of time appears to be a common endocrine factor in both premenopausal and menopausal women who develop breast or endometrial cancer.

A number of observations indicate that estrogen target tissues are exposed primarily to estrone and not estradiol when the former is exclusively produced. First, our studies and the work of others has shown that the conversion of estrone to estradiol in the circulating blood is limited, being in the order at 5 - 10% [36]. Secondly, measurements of the levels of estrone and estradiol in postmenopausal women have shown that the concentration of circulating

estrone is 3 - 5 times higher than that of estradiol [37,38]. Also, it is important to note that the affinity of the plasma sex steroid binding globulin (SBG) for estrone is much lower for estrone than for estradiol [39]. Therefore, the amount of free estrone available to target cells may actually be several hundred fold higher than that of estradiol. Thirdly, the work of Gurpide has shown that estrone and estradiol enter normal endometrial tissue equally well and further, that the intracellular steady state concentration of estrone is higher than that of estradiol [40]. Earlier, Pearlman *et al.*, demonstrated that estrone is the predominent estrogen in plasma and in normal or cancerous breast tissue when long-term infusions of radioactive estrone were carried out in women with carcinoma of the breast [41]. More recently, Korenman reported that estrone is the major estrogen present in cytosol obtained from those human breast tumors which contained estrogen receptors and therefore, are presumed to be estrogen dependent [42]. If endogenous estrogens play any role in the initiation and/or stimulation of cancer of the breast or endometrium in the human, it would appear that the offending estrogen most often is estrone. However, it is by no means clear that estrone *per se* has unusual estrogenic or carcinogenic properties. Indeed the bulk of the available evidence suggests that estrogens do not differ qualitatively either in their ability to stimulate normal tissues or to promote tumors.

The exposure of target cells to estrogen in anovulatory and postmenopausal women differs in two major respects from that which occurs in normally cycling females. First, peripheral estrone production is relatively constant rather than cyclic and second, target cells are not subjected to the modifying effect of progesterone. Both may be important in determining the rate of proliferation of target cells and therefore the likelihood of a neoplastic transformation. Recent studies by Gurpide suggest that progestin administration reduces estrogen receptor levels in human endometrium [43]. Also, Milgrom *et al.* [44] showed that progesterone administration to estrogen primed guinea pigs lowered the concentration of uterine progesterone receptor. It is not clear whether this effect is at the level of cytoplasmic estrogen receptor synthesis or replenishment but it is consistent with the fact that endometrial estrogen receptor levels are much lower in the luteal than in the proliferative phase of the menstrual cycle [45]. The ability of progestins to induce regression of well-differentiated endometrial carcinoma may thus be explained by decreased levels of estrogen receptor. Experimental evidence from studies in rabbits [46] indicate that progestins can inhibit carcinogenic induced endometrial cancer under some circumstances and not others. Administration of progestins markedly reduced tumor development in response to methylcholanthrene impregnated strings inserted in normal animals whereas no effect was observed in estrogen treated castrate animals. However, pharmacologic doses of estrogen were administered and the dose of progestin may have been too low to overcome the estrogen effect. These results suggest that inhibition of estrogen promoted cancer of the uterus may depend on a critical ratio of progesterone to estrogen. It is unfortunate that most studies of the role of steroids in tumorigenesis or tumor growth regulation are done with pharmacologic doses of single hormones.

It is obvious that much more work is needed before the role of the sex steroids and other hormones in cancer development can be elucidated. It is also evident that an understanding not only of the secretion of estrogens and their interaction with target cells, but also of the metabolic factors which regulate peripheral estrogen synthesis is essential to progress towards this goal.

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DISCUSSION

Kellie. Do you imply that estrone is binding to the receptor. Or that estrone is convered to estradiol which is then bound to the receptor?

Siiteri. I imply that when there is only estrone being produced as in postmenopausal women the ratio of estrone to estradiol in the circulation is about 5:1. Under these circumstances the concentration of estrone is indeed high enough to interact with cytoplasmic receptor and that the estrone receptor complex is capable of eliciting a response at the level of the nucleus as shown by Gorski and his associates.

McGuire. Let us reconsider an earlier question. You wish the audience to believe that progesterone acts by blocking estrogen receptor synthesis. Actually, if one measures estrogen receptor in uteri before and after progesterone administration, very little happens to the receptor level. Progesterone can interfere with the full replenishment of estrogen receptor but even there, there is sufficient receptor present in the progesterone treated cell to be able to be translocated by physiological amounts of estrogen and achieve a maximal biological response. I would conclude that progesterone might be acting by some other mechanism.

Siiteri. You may be absolutely correct insofar as the immature rat model is concerned. However, Dr. Gurpide has shown that progestin administration to women decreases estrogen receptor levels in the endometrium. Also, if you look for estrogen receptor in human uterus during the luteal phase by sucrose gradient examination, it's very difficult to demonstrate its presence at all. There may be a species difference here, but clearly I am basing my remarks on human studies.

McGuire. Let us consider human studies then. Dr. Gurpide showed a slide indicating a 50% reduction in estrogen receptor following pharmacological doses of provera. It would seem to me that the rat is very similar to the human in this regard.

Lidner. I wonder whether these statements of progesterone antagonism of estrogen action in target cells are not generalizations. It may depend on what target cells you are talking about. In the rat uterus for instance, it was shown in our laboratory that your model applies perfectly for the epithelial elements of the uterus where estrogen alone has a stimulatory effect which is suppressible by progesterone. But if you look at the stromal element of the endometrium there is no effect of estrogen alone or either DNA synthesis or RNA synthesis and what not. But if you pretreat this with progesterone then estrogen is capable of acting on these stromal elements and cause nucleolar development, DNA and RNA synthesis. So in some situations the two seem to be similar.

Siiteri. You are correct.

Lidner. By a model of silastic capsules, can one really take this as evidence for the immunosuppressive effect of progesterone or could this be antiinflammatory action when you look at the glandular reaction?

Siiteri. Well, I would say that our silastic capsule model does demonstrate antiinflammatory activity of progesterone. However, we can in fact show suppression of human mixed lymphocyte cultures by progesterone which is not very different from that which is produced by cortisol. This is not a cytotoxic effect but is an ability to depress the response of T-lymphocytes to foreign antigens.

Johnsen. I was a little unhappy that you had to skip the last part of your lecture. May I ask you a question about this. I understand that you think that 5-α-reduced C-19-steroids inhibit the conversion of androstenedione to estrone. You than state in yhour abstract the following: "It is of interest in this regard that the production of adrenal precursors of 5-α's and the 5α/5ß ratio is reduced in women with breast cancer and men with lung cancer." I have 2 questions about this. First, what is the adrenal precursors of 5-α's? I don't know it. I know that DHA, androstenedione, testosterone and so on all come out in the urine as both 5-α and 5-ß, but that is not a question of produc-

tion but of metabolism in the liver, and here of the concentrations of 5-α reductase and 5-ß reductase. Secondly, I am quite willing to accept that in cancer patients the 5-α/5-ß ratio is low. But this I think is completely non-specific because all kinds of severely ill patients have low ratio irrespective of the type of disorder. Again, this is a question of steroid metabolism in the liver, not of production. Am I right?

Siiteri. The point I was going to get to if I had time was that 5α-reduced steroids have the capacity to inhibit the aromatase enzyme. This has been established in our and Dr. Brodie's laboratory. I suggest that the 5α/5ß ratio may reflect the relative amount of 5α steroid produced which may, in fact, be a regulatory factor in peripheral production of estrogen from androstenedione. We think there may be an inverse relation between the amount of 5α-reduction and the efficiency for conversion of androstenedione to estrogen. The early observation of Dr. Bulbrook showing a low 5α/5ß ratio in patients with a high incidence of breast cancer has not had any biological explanation that I know of.

Clark. With respect to the depletion of cytoplasmic estrogen receptors, one should not forget the work of West and Brenner. They have infused estradiol and progesterone in the monkey in order to simulate the blood levels which are observed during the normal menstrual cycle. With this model system, they observe a depletion of cytoplasmic estrogen receptors under the influence of progesterone. We have measured replenishment in the endometrium and myometrium and it occurs equally in both places. This does not rule out differential replenishment in some cell types but it does make it less likely. Our recent work has shown that not only does progesterone lower the quantity of cytoplasmic estrogen receptor but it also decreases the length of time that the complex stays in the nucleus; i.e., decreases nuclear retention. Thus, progesterone's action may be two-fold: (a) to decrease the cytoplasmic concentration and thereby decrease the probability of estrogen receptor binding and (b) to decrease the ability of the receptor estrogen to complex to remain in the nucleus and thus to reduce its ability to stimulate estrogen directed uterotropic responses.