

## **Serum Concentrations of Estrone, Androstenedione, Testosterone and Sex-hormone-binding Globulin in Postmenopausal Women with Breast Cancer and in Age-matched Controls**

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### ABSTRACT

The concentrations of estrone (E1), androstenedione (A), testosterone (T) and sex-hormone-binding globulin (SHBG) in the serum were determined in 122 postmenopausal women, unselected with respect to age and stage of disease and with a newly diagnosed breast cancer. The results were compared with those in 122 age-matched women without breast cancer, selected from the population register. The patients were found to have a significantly higher mean level than the controls of E1 (132 and 108 pmol/l), A (2.5 and 1.6 nmol/l) and T (1.54 and 1.38 nmol/l) and a lower level of SHBG (40.2 and 47.3 nmol/l) in the serum. A multiple regression analysis revealed in the control group that the serum level of E1 was significantly correlated to A ( $r=0.48$ ,  $p < 0.001$ ) and T ( $r=0.45$ ,  $p < 0.001$ ). In the patient group E1 was only slightly correlated to T ( $r=0.25$ ,  $p < 0.01$ ) and not to A ( $r=0.10$ ,  $p > 0.05$ ). A significant negative correlation was found between SHBG and weight in both groups. Otherwise no significant correlations were found between any of the hormone levels and age, stage of disease or weight.

It was concluded that an increased availability of A and T, leading to an increased androgenic stimulation - and therefore decreased SHBG - and an increased E1 level, is the most reasonable explanation for the findings. The lack of correlation between E1 and A in the patient group is however difficult to explain and the results do not seem to fit into a definite hypothesis.

### INTRODUCTION

Epidemiological and clinical evidence that the risk for breast cancer is influenced by endocrine factors (40) has initiated numerous studies designed to define a specific endocrine environment that predisposes to breast cancer. Major interest has been paid to the urinary excretion of androgen and estrogen metabolites. Although contradictory in many instances, the results from these studies have initiated several hypotheses and two of them have attracted special interest.

According to the first of them a decreased urinary excretion of etiocholanolone and androsterone was associated with an increased risk of breast cancer and in the manifest disease with a poor prognosis and a poor response to endocrine ablative surgery (cf. 19). Contradictory results have however been reported (cf. 62) and the androgen excretion has also been shown to be decreased as a nonspecific consequence of illness (61).

Secondly, the "estriol hypothesis" was derived from the fact that carcinogenic potential has been demonstrated for estrone (E1) and estradiol (E2) in experimental research but not for estriol (E3) and that E3 has the capacity to impede certain estrogenic activities of E1 and E2 (33). The relative amount of E3 in relation to E1 and E2 was therefore suggested as an important determinant for breast cancer risk. This hypothesis was supported when population studies revealed a parallelism between the urinary estriol ratio and a high, intermediate and low breast cancer risk, respectively (13, 41). The difference was most marked in young women, a finding combined with the previous finding of a reduced breast cancer risk due to an early age at first birth (40).

Recent research has revealed several lines of data which has seriously brought into challenge the concepts that the pattern of excretion of the urinary androgens and estrogens is causally related to the etiology of breast cancer (cf. 32, 62). Results derived from determinations of the steroid hormones in serum are very few and have not resulted in any convincing alternative hypothesis (32).

The design of the present investigation was not primarily aimed at analysis of steroid hormones and their binding globuline. The availability of serum within the frame of an ongoing epidemiologic breast cancer study which included an assessment of thyroid function and an access to recently developed radio-immunoassays did, however, enable the study. The aim was therefore to compare the serum concentrations of E1, androstenedione (A), testosterone (T) and sex-hormone-binding globulin (SHBG) in an unselected series of postmenopausal women with a newly diagnosed breast cancer with those in a group of age-matched, non-hospitalized, postmenopausal women without breast cancer.

#### SUBJECTS AND METHODS

##### Patients

This study was based on 149 postmenopausal women included in a series of 179 women with breast cancer registered consecutively during five months in four Swedish counties. The population within this area was uniform as to race and nationality. Only two of the breast cancer patients who were diagnosed during the observation period (2/181) refused therapy and participation in the study.

Hospital records were written on a special form and all patients answered a comprehensive questionnaire concerning their reproductive history, gynecologic

diseases, height, weight, drug consumption and other factors of epidemiologic interest. All patients were classified according to the TNM-classification (23) (Table 1).

Two blood samples were drawn for analysis of SHBG and hormones in the serum. The sera were directly sent to Uppsala and arrived the same evening or the next morning and were frozen and stored at  $-90^{\circ}\text{C}$  until analyzed. The first serum sample was taken after admission to the hospital but before operation and was used for the determination of follicle stimulating hormone (FSH) and for the assessment of thyroid function. The second serum sample was drawn randomly between 09.00 am and 04.00 pm more than one week postoperatively, the median time being three weeks and three quarters of the samples taken within six weeks. The second serum sample was used for analyses of the steroid hormones and SHBG.

Table 1. Staging according to the TNM classification

TNM-classification		
Category	Number	%
I	52	37
II	68	48
III	13	9
IV	8	6
	141	100

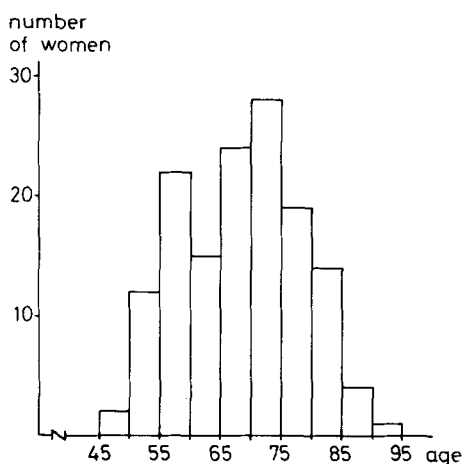


Fig. 1. Age distribution of 141 patients and corresponding controls.

### Controls

The control group consisted of age-matched women without breast cancer. In this study those 151 within the total control group of 179 women who were postmenopausal were included. They were selected from the computerized population register within each county where the women closest in age to each respective breast cancer patient was chosen. Control women who refused to participate (25=14 %) were replaced by an alternative control selected in the same way as the first one. As a result each breast cancer patient had one age-matched control with not more than 3 days age difference to the corresponding patient.

All women in the control group answered a questionnaire identical to that in the patient group. In addition they were all examined at the office of their district medical nurse by one of us (HOA). At the examination blood samples for analysis of hormones in serum were drawn and centrifuged. This occurred, as in

the patient group, randomly between 09.00 am and 04.00 pm. The sera were directly sent to Uppsala in the same way as in the patient group and were frozen and stored at  $-90^{\circ}\text{C}$ . The subsequent analysis included, as in the patient group, FSH and SHBG in addition to the steroid hormones.

#### Selection of the postmenopausal group

When comparing the age-matched pairs both the patient and the corresponding control were postmenopausal according to history and an increased concentration of FSH in the serum (60) in 141 instances. The age distribution of this group is shown in Fig. 1., the average and the median age being 68 years. In these groups two patients and one control had to be excluded due to estrogen treatment. In the remaining group postoperatively taken serum samples were available in only 122 patients. With respect to the factors relevant for the investigation, the loss can be considered random and the 122 pairs analyzed thus representative for the whole postmenopausal groups.

#### Radioimmunoassays

Testosterone: An antibody raised in sheep against testosterone-3- $\alpha$ -carboxy-methyl bovine serum albumin was used (gift from Dr. Lars-Erik Edqvist, Royal Veterinary High School, Uppsala, Sweden). The antibody crossreacts 56 % with dehydrotestosterone and 7 % with androstenedione but less than 1 % with dehydroepiandrosterone and estradiol. It was used in a 1:15 000 dilution with ethanol. As tracer 1,2,6,7- $^3\text{H}$ -testosterone with a specific activity of 85 Ci/mmol purchased from the New England Nuclear, Boston Massachusetts, USA was used. It was diluted to give approximately 85 pg per assay tube. The procedure and reagents of the assay were the same as described previously (14). 0.4 ml aliquots of plasma were extracted with 4 ml of diethyl ether for the assay. A blank of 55 pg (CV 21 %) was found in charcoal treated plasma at this extraction volume and results were corrected for this plasma blank. The within and between assay coefficients of variation were found to be 14 and 18 %, respectively.

Androstenedione: A previously described antibody raised in adult ewes against androstenedione-3-oxime human serum albumin (gift from Dr. Guy E. Abraham) was used. The antibody crossreacts 25 % with dehydroepiandrosterone (2). It was used in a 1:500 dilution. 1,2,6,7- $^3\text{H}$ -androstenedione with a specific activity of 40 Ci/mmol purchased from New England Nuclear was used as tracer. It was diluted to give approximately 72 pg per assay tube. The procedure and reagents of the assay were the same as described previously (14). 0.1 ml aliquots of serum were extracted with 1.5 ml of diethyl ether for the assay. A plasma blank of 1.03 pg (CV 20 %) was found in charcoal treated plasma at this extraction volume and the results were corrected for this blank. The within and between coefficients of variation for the assay were found to be 10 and 20 %, respectively.

Estrone was determined by radioimmunoassay as described by Axelsson et al. (7).

Sex-hormone-binding globulin was assayed by the ammoniumsulphate precipitation technique described by Rosner (50) and the results are expressed as the dihydrotestosterone-binding capacity of plasma in nmol/l.

#### Calculations and statistical methods

As an index of obesity independent of height we used the index of Quetelet (weight/height<sup>2</sup>) which was shown to fulfill important criteria for such an index (29). As the SHBG, E1 and T showed a skewed distribution their logarithmic values were calculated. This made the normal distribution assumption of the t-test for matched groups more plausible.

The correction for the relatively high plasma blank of A by subtraction of its mean value gave some negative results. If these values had been discarded the mean estimate of A would have become positively biased. They were therefore included in the calculation which, however, made the use of logarithmed values impossible. Due to the skewed distribution of A Wilcoxon's nonparametric matched-pairs signed-ranks test was used as a complement to the t-test. In the calculation of the confidence intervals of the population means of A the standard deviation of the blank had to be included.

The product moment correlation coefficient with the corresponding p-value of the independence hypothesis was used as a measure of correlation. A multiple regression analysis was also applied to evaluate how much of the variance in E1 could be explained by variance in other "independent" variables.

All values refer to two-tailed tests. When values were missing in the patient or the corresponding control, both were excluded from the calculations for that variable.

#### RESULTS

Estrone (E1). The mean value in the patient group (132 pmol/l) was significantly higher ( $p < 0.01$ ) than the mean value in the control group (108 pmol/l) (Table 2). There was a wide range of variation in both groups (Fig. 2) but values exceeding 200 pmol/l were found in only 15 controls compared to 29 patients. The means calculated on arithmetic values were 152 pmol/l (41.2 pg/ml) in the patient group and 124 pmol/l (33.5 pg/ml) in the control group.

Androstenedione (A). The distribution was slightly shifted to higher values in the patient group (Fig. 3) whose mean value after correction for the blank was 2.5 nmol/l (0.71 ng/ml) which is significantly higher ( $p=0.009$ , t-test;  $p=0.006$ , Wilcoxon's test) than the mean value in the control group of 1.6 nmol/l (0.46 ng/ml) (Table 2). Values lower than the mean value of the blank (3.7 nmol/l) were found in 16 patients and 22 controls.

Testosterone (T). Even the distribution of testosterone was shifted to

Table 2. Results concerning serum concentrations of estrone (E1), androstenedione (A), testosterone (T) and sex-hormone-binding globulin (SHBG) in the patient and the control group calculated on arithmetic values for A and logarithmic for E1, T and SHBG

Tests	Mean and 95 % confidence limits of the mean		Median		p-value	
	patients	controls	patients	controls		
E1 pmol/l	132	112 - 145	108	98 - 119	111	0.004
A nmol/l	2.5	1.9 - 3.1	1.6	1.1 - 2.1	1.3	0.009
T nmol/l	1.54	1.43 - 1.65	1.38	1.28 - 1.50	1.39	0.042
SHBG nmol/l	40.2	36.8 - 43.9	47.3	44.0 - 50.9	50.9	0.006

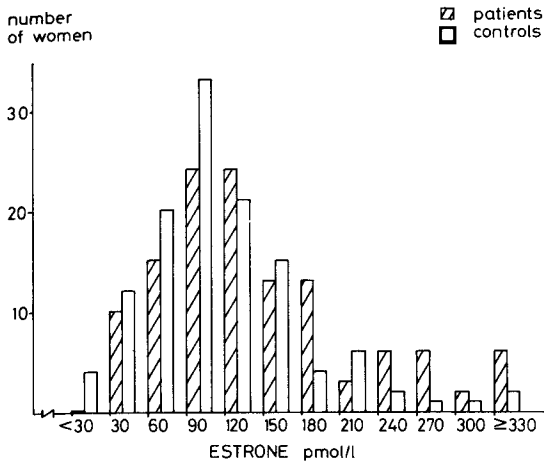


Fig. 2. Distribution of serum levels of estrone in patient and control group

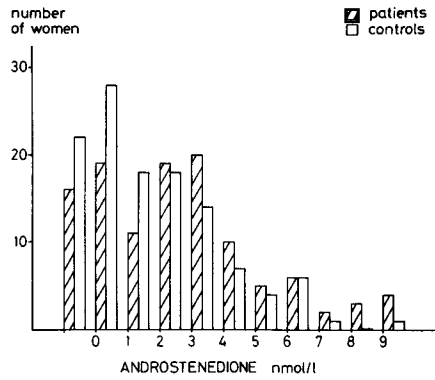


Fig. 3. Distribution of serum levels of androstenedione in patient and control group

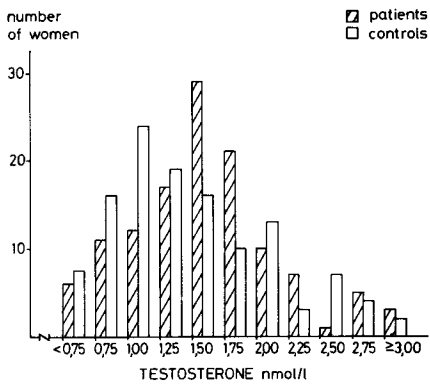


Fig. 4. Distribution of serum levels of testosterone in patient and control group

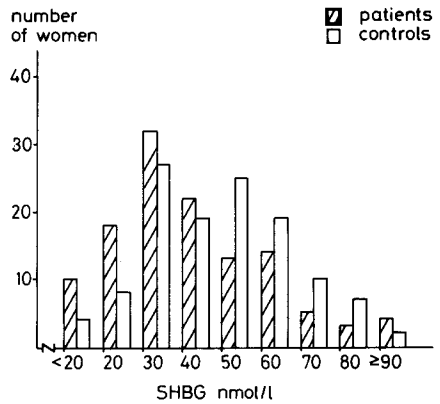


Fig. 5. Distribution of serum levels of sex-hormone-binding globulin in patient and control group

higher values in the patient group (Fig. 4) but the difference in mean values between the groups was very small albeit significant ( $p < 0.05$ ) (Table 2). The skewness of the distributions (Fig. 4) was less pronounced than for E1 (Fig. 2) and A (Fig. 3) and the arithmetic means - 1.65 nmol/l (0.48 ng/ml) and 1.52 nmol/l (0.44 ng/ml) - close to the logarithmic.

Sex-hormone-binding globulin (SHBG). In contrast to E1, A and T this distribution was shifted towards lower values in the patient group (Fig. 5). The difference is highly significant ( $p < 0.01$ ). Due to some very high values in the patient group (Fig. 5) the mean values - 40.2 nmol/l and 47.3 nmol/l - differ less than the median values - 40.1 and 50.9, respectively (Table 2). The arithmetic means were 45.2 and 50.8 nmol in the patient and control groups.

Relations to age, weight and stage of disease. Subgrouping of the material according to age revealed no obvious trend in the differences between patients and controls for any of the variables. Thus in all decades from 45 to 75 years or more the patients, on the average, had a slightly higher A and T concentration in the serum. E1 concentration did not differ between patients and controls aged 45-54 years but the patients had higher mean values in all subsequent age-groups. The patients had a lower mean SHBG value in all groups except in that including women over 75 years of age. Not any of the variables was significantly correlated to age, either in the control or in the patient group (Table 3).

Table 3. Correlation coefficients to age, weight, Quetelet's index and stage of disease according to the TNM-classification

Test	Group	Correlated to			
		age	weight	Quetelet's index <sup>1/</sup>	TNM classification
E1	patient	0.01	0.05	0.07	0.04
	control	0.07	0.14	0.22 <sup>2/</sup>	-
A	patient	0.00	0.07	0.09	-0.08
	control	-0.01	0.03	0.00	-
T	patient	-0.11	-0.04	-0.03	-0.01
	control	-0.09	0.01	-0.05	-
SHBG	patient	0.13	-0.28 <sup>3/</sup>	-0.31 <sup>4/</sup>	0.01
	control	0.12	-0.29 <sup>3/</sup>	0.34 <sup>4/</sup>	

1/ Quetelet's index = weight/height<sup>2</sup>

2/  $p < 0.05$

3/  $p < 0.01$

4/  $p < 0.001$



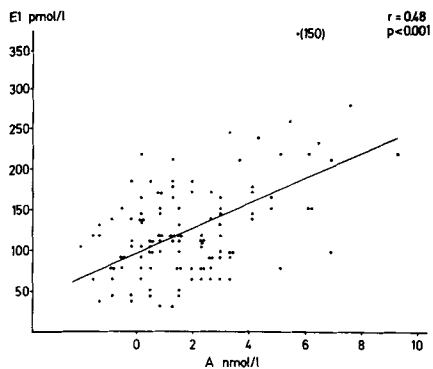


Fig. 6. Correlation between serum levels of estrone and androstenedione in the control group

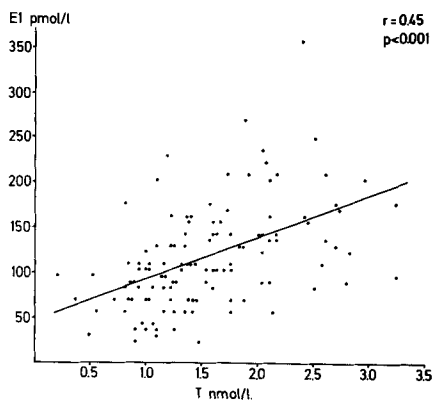


Fig. 7. Correlation between serum levels of estrone and testosterone in the control group

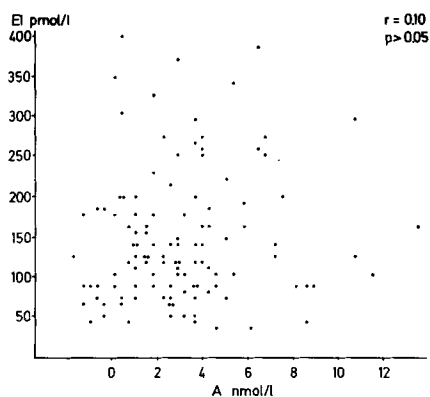


Fig. 8. Correlation between serum levels of estrone and androstenedione in the patient group

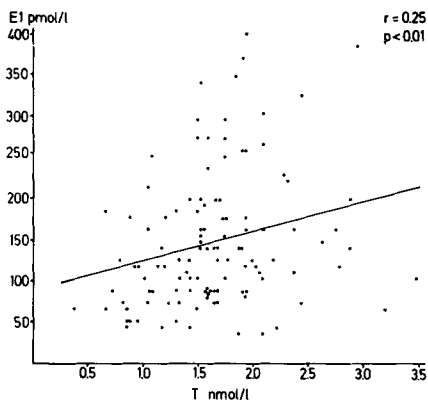


Fig. 9. Correlation between serum levels of estrone and testosterone in the patient group

Weight varied from 41 to 110 kg with a close accordance between the distributions and a mean weight of 65.55 kg in the patient group and 65.75 kg in the control group. The same accordance was found concerning height with a range from 140 to 178 and mean values of 161.7 and 161.4 cm, respectively. The differences were, as reported elsewhere (4), not statistically significant in either weight, height or the Quetelet's index.

When weight and the index for overweight (Quetelet's index) were correlated to the steroid hormone levels the correlation coefficients were very low (Table 3). Except for a low degree of significance ( $p < 0.05$ ) for the correlation between E1 and Quetelet's index in the control group (Table 3) they were all insignificant ( $p > 0.05$ ). The same was true in the patient group in the correlation to the stage of the disease according to the TNM classification (Table 3). A slight but significant negative correlation was found between Quetelet's index and the SHBG, in both groups (Table 3).

Interrelations between E1, A and T were finally analyzed with calculation of correlation coefficients and with a multiple regression analysis using E1 as a dependent variable and A and T as independent variables in addition to age and the weight index.

In the control group there was a highly significant correlation between E1 and A ( $r=0.48$ ) (Fig. 6) and E1 and T ( $r=0.45$ ) (Fig. 7). The situation was quite different in the patient group where no significant correlation ( $r=0.10$ ) was found between E1 and A (Fig. 8) and a low correlation ( $r=0.25$ ) between E1 and T (Fig. 9).

According to the multiple regression analysis the variances in the serum level of E1 could be explained to 14 % and 58 % in the patient and control group, respectively, by variations primarily in A and T with very little contribution to the determination coefficient from age and the weight index. SHBG was not significantly correlated to E1, A or T in any of the groups ( $r=0.17$ ).

#### DISCUSSION

The estrogen and androgen metabolism and the serum concentration after the menopause differ in many aspects from the premenopausal state. The serum concentrations of estradiol and estriol were thus generally found to be low (8, 15, 16, 17, 27, 35, 46) while estrone is the dominant estrogen in the postmenopause with normal values in most reports which were in agreement with the mean value in our control group (17, 25, 27, 35, 42, 46) and in some reports even higher (3, 9, 58). Also concerning testosterone our study revealed a mean value in accordance with previous authors (3, 10, 24, 30, 58) although lower (25, 42) as well as higher (16) normal values have been reported. Our mean value for androstenedione in the control group when corrected for the blank, is in the range found by some other investigators using the same antibody for the radioimmuno-

assay (2, 3, 42) but somewhat lower than that reported by others who usually found values in the interval 0.75-1.09 ng/ml (16, 17, 25, 58).

The use of non-hospitalized controls made a comparability with the patient group with regard to a recent operation impossible. Surgical trauma has been shown to induce a small and transitory fall of A and T but no change of E1 in normal women (28). The fact that these changes were normalized within one week (28) and that our patients had higher mean values than the controls seems to exclude postoperative changes as a cause of the differences found in this study. Diurnal variations of A and T in women were shown by Vermeulen (58) whereas results concerning E1 have been contradictory (8, 58). A significant difference in the average sampling time between patients and controls was, however, considered unlikely and the changes during the day too small (58) to account for the differences found between the groups concerning A and T.

Accumulated evidence now support the view that negligible amounts of estrogens are secreted by the postmenopausal ovaries or adrenals (12, 16, 25, 36-38, 43). Instead most estrogens after the menopause are derived from the peripheral conversion of the androgens (17, 34, 37) secreted by the ovaries and adrenals (16, 17, 25, 26, 36). Thus E1 can be accounted for nearly exclusively by the peripheral conversion of A whereas the production of E2 in the blood is mainly due to the conversion of T both directly and indirectly via A and E1 (34, 38). Therefore the higher mean concentration of E1 found in women with breast cancer in this study has to be discussed in terms of a lowered metabolic clearance rate or an increased production as a consequence of either increased availability of plasma precursors or of an increased extent of conversion of these precursors to the product hormone (39). The metabolic clearance rate of estrogens (E1, E2, E3) has been shown to be decreased in postmenopausal women compared to premenopausal (15, 35) but in breast cancer patients there has rather been a tendency to higher metabolic clearance of E1 (31) and even A (31, 45) than in normals.

The conversion rate of androstenedione to estrone in breast cancer patients did not differ from that in normal controls (31, 45). But in women with endometrial carcinoma (18, 53) and endometrial hyperplasia (53) an increased conversion was found and suggested an important etiologic factor (39). These findings might imply that the principal reason for the higher estrone level in the breast cancer group is an increased availability of the precursors, mainly A and T. This concept was supported by the significantly higher values found in the patient group in our study (Table 2). A slight difference in the same direction was also suggested for androstenedione in a recent report by Rose et al. (48). Increased testosterone levels were also recently found in breast cancer patients by McFadyen et al. (44) and Sarfaty et al. (51) although two previous reports are in disagreement with this finding (22, 56).

Whereas in the control group a considerable part of the estrone variations

were related to the availability of precursors (Figs 6 and 7), the lack of correlation between E1 and A and the low correlation between E1 and T in the patient group are confusing and suggest that other factors have an influence on the serum estrone level. The results of Poortman et al. (45) and Kirschner et al. (31) are also inconsistent with the concept of an increased availability of precursor hormones in breast cancer patients as they found no difference in the androstenedione production rate compared to age-matched controls.

Although the production of A and T can be accounted for exclusively by adrenal and ovarian secretion and interconversion between these hormones (58) an additional source of estrogen was suggested by Hill et al. (21). They demonstrated the production of estrogens by bacteria from the biliary steroids related to the fat content in the diet. This could then link the observed great international variations in breast cancer incidence - obviously related to environmental factors (4) - to an endocrine hypothesis.

The distribution and mean value of SHBG in this material is in the range previously reported in normal women (49). Values in breast cancer patients are not available for comparison. Serum SHBG levels are known to be regulated by the biological active, unbound hormone fraction with the androgens having an inhibitory and estrogens a stimulatory influence (6, 57). Thyroid function, assessed in this material by determination of TSH, T3, rT3, T4 and T3-resin uptake and reported elsewhere (5), is also known to influence the SHBG level (6) and the steroid hormone metabolism (54). The results were, however, mainly within the limits of the reference range and no significant correlations to SHBG, E1, A or T were found. The lower concentration of SHBG in the patient group speaks against increased estrogenicity in breast cancer patients and might rather indicate a predominance of androgenic influence (6) consistent with the concept of increased production of A and T.

There is a well-known relation between the biological activity of steroid hormones and their binding to SHBG. Consequently the binding of testosterone is high and that of androstenedione and estrone very low or negligible (6, 49). A lower SHBG level in the patient group will therefore primarily affect testosterone and cause an increased fraction of the unbound hormone. An additional consequence would be an increased metabolic clearance rate of testosterone (11, 57) which could diminish the influence of an increased production rate on the peripheral concentration (1).

The ability of adipose tissue to metabolize C19 steroids to estrogens has been demonstrated in vitro (52). A positive correlation was also found between weight and conversion rate of A to E1 in normal postmenopausal women (53) and in women with endometrial cancer (47). The lack of significant correlation between weight and serum estrone levels in normal postmenopausal women found in the present study, is in accordance with the findings of Judd et al. (25, 27) but a

positive correlation was found in women with endometrial carcinoma (27). Our study did not reveal any difference in weight or in different weight indices between patients and controls (4) and no correlations to E1, A or T except for a low correlation between E1 and Quetelet's index in the control group. If the amount of fat tissue is positively correlated to the conversion rate of A to E1, an influence on the serum levels might be obscured if the body weight is negatively correlated to the percentage of E1 derived from plasma A which enters the circulation, as noticed by Grodin et al. (17). But if obesity was a risk factor for breast cancer and the amount of fat tissue quantitatively influenced the aromatization of estrogen precursors, then the conversion rate of A to E1 ought to be increased in breast cancer patients. This was, as pointed out above, not confirmed by Poortman et al. (45) or by Kirschner et al. (31) who found a conversion rate in close agreement with other studies in normal postmenopausal women (37, 53). A negative correlation between SHBG and obesity, confirmed in this study, was previously noticed by Vermeulen et al. (57).

The conversion rate of A to E1 has repeatedly been found to be 1.2-1.3 % in premenopausal women (37, 53) and at least two times higher in postmenopausal women (17, 37, 38, 45, 53). The concept of a progressive increase with advancing age, as suggested by Hemsell et al. (20) has less support and was not confirmed in women with endometrial carcinoma (47). It is also in disagreement with the lack of correlation between age and excretion of urinary estrogens found by Thijssen et al. (55) in normal postmenopausal women. Neither was there any correlation between age and serum levels of E1 or A in the present study nor in those by Judd et al. (25, 27).

In conclusion, serum concentrations of E1 and SHBG have, to our knowledge, not been studied before in breast cancer patients and thus the increased E1 and decreased SHBG never observed, whereas the increased A and T levels have some support in a few recent studies. Although an increased production of estrogen precursors seems to be the most reasonable explanation for these findings, they are difficult to fit into a definite hypothesis and uniform support for this view was not perceivable in earlier studies. The significance of these findings needs therefore further confirmation, primarily by studies of steroid hormone kinetics including production, conversion and metabolic clearance in breast cancer patients and comparable controls.

#### ACKNOWLEDGMENT

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