The endogenous concentrations and the subcellular distribution of dehydroepiandrosterone (DHEA) and 5-androstene-3β,17β-diol (ADIOL) were measured in malignant and nonmalignant human breast tissue from both pre- and postmenopausal women. DHEA 3-sulfate was measured only in the cytosol. A greater tissue-plasma gradient of DHEA was present with large variations. The highest concentration of DHEA and ADIOL occurred in the nuclear fraction (average, 2.9 and 1.6 times the concentration in cytosol). With respect to DHEA, this finding is remarkable because no specific binding protein in human breast tissue has been reported. The concentration of DHEA 3-sulfate was significantly higher in the cytosol of nonmalignant than in malignant breast tissues. No significant differences in tissue concentrations of DHEA and ADIOL were found in malignant and nonmalignant breast tissue. The concentration of estrogens was measured in the cytosol and the nuclear fraction of the same tissues, as reported in a previous paper. We found a significantly higher estradiol concentration in malignant tissue as compared to nonmalignant tissue. When the ratio of ADIOL to estradiol was calculated from the combined data, a significant difference was found only in the cytosol of premenopausal cancer patients versus normal women. No difference was seen in the postmenopausal women. No difference in the ADIOL:estradiol ratio was found between normal and malignant breast tissue of patients of the same menopausal status.

INTRODUCTION

The early work of Bulbrook et al. (5–7) on the subnormal urinary excretion of androgen metabolites in women who later developed breast cancer stimulated many studies on the production, excretion, and metabolism of androgenic hormones in mammmary cancer patients. On the basis of these studies, several hypotheses on the involvement of androgens in breast cancer have been made. Adams et al. (1) reported that both DHEA and ADIOL inhibited estradiol sulfotransferase in breast tissue, which controls the rate of sulfurylation of DHEA and which is correlated with prognosis and response to adrenalectomy (8). Poortman et al. (9) showed that an imbalance between estrogens and androgens (i.e., lowered levels of ADIOL but unaltered levels of estradiol might favor an unopposed estrogenic stimulus. To test this hypothesis, the concentrations of estrone, estradiol, DHEA, DHEA-S, and ADIOL were measured in the cytosol and in the nuclear fractions of human primary breast tumor tissues and of nonmalignant breast tissues from pre- and postmenopausal women. The data on the estrogen concentrations in these tissues are reported in the accompanying paper (10). In this paper, we have investigated the androgen concentrations and the ADIOL:estradiol ratio in the same tissue samples.

MATERIALS AND METHODS

Tissue Specimens. Androgens were measured in the same cytosols and nuclear fractions of the 113 breast tissue specimens in which we measured the estrogen content (11). The tissues were classified on the bases of the histology of the tissue as judged by a pathologist and the menopausal status in 5 groups outlined previously (11). The characteristics of the 5 groups of women are given in Table 1.

Analytical Procedures. Cytosols and nuclear fractions were prepared by centrifugation of the tissues homogenized at 4°C for 30 min at 100,000 x g as described previously (12). Estradiol receptor sites were measured according to a modified European Organization for Research on the Treatment of Cancer procedure (13). DHEA and ADIOL were extracted from high-speed (100,000 x g) cytosol and nuclear fractions with ethanol:aceton (1:1, v/v) (14). This nuclear fraction also contained microsomes and mitochondria. In separate experiments, we found that at least 75% of the total amount of androgens in the 800 x g pellet, consisting predominantly of nuclei. The extracted steroids were purified on a column of Celite:ethylene glycol (5:3, v/v), with increasing amounts of ethyl acetate in isooctane. This extraction removes over 95% of the steroid hormones from the subcellular fractions (15). After separation of DHEA and ADIOL on Celite:ethylene glycol columns, both steroids were measured in duplicate, DHEA in 2 dilutions, by radioimmunoassay using highly specific antisera (16). DHEA-S was estimated directly only in the cytosol without purification, using a kit from Radioassay Systems Laboratories, Inc., Carson, CA. Results for both cytosol and nuclear fractions are expressed per g, wet weight, of tissue. Cytosol protein concentration was measured according to the method of Lowry et al. (17) using bovine serum albumin as standard. Radioactivity was measured by adding 10 ml of a scintillator (Instagel; Packard Becker BV-Chemical Operation, Groningen, The Netherlands) to the samples. Liquid scintillation counting was performed with a Packard Model 2660 liquid scintillation counter with automatic correction for quenching.

Statistical Analysis. The individual data per group were not normally distributed. Therefore, differences between the groups were tested for significance by the 2-tailed Wilcoxon rank sum test. Correlations were calculated by the Spearman coefficient of correlation.

RESULTS

Intracellular Concentrations in Relation to Normal Plasma Levels. The concentrations of DHEA in cytosol an nuclear frac-
tions and total tissue (cytosol plus nuclear fractions) are shown in Chart 1. Tissue concentrations showed large variation; 95% of the values in total tissue concentrations ranged from 5 to 500 pmol/g tissue. Median values for each individual group are indicated in Chart 1. In all groups, in approximately one-half of the tissue specimens (except for both carcinoma Groups 2 and 4 and for the hypertrophic tissues in Group 5, as far as the concentration in the cytosol is concerned), the concentration of DHEA in the cytosol, in the nuclear fraction, and in total tissue (pmol/g) is much higher than the plasma levels (pmol/ml) of healthy volunteers. In all groups except Group 3, the median values are higher in the nuclear fraction than in the cytosol.

The tissue concentrations and the subcellular distribution of ADIOL are shown in Chart 2. ADIOL concentrations were much lower than for DHEA but also showed a large variation; 95% of the values ranged from 0.3 to 30 pmol/g tissue. In most of the tissue samples, the concentration of ADIOL in the cytosol, nuclear fractions, and the tissue were within the range of normal plasma levels. Consequently, the tissue:plasma gradient for ADIOL is lower than for DHEA. Median concentrations of ADIOL were higher in the nuclear than in the cytosol fractions, except for Groups 3 and 4 and for the hypertrophic tissue in Group 5 where the median concentrations are similar.

The concentrations of DHEA-S in cytosol are shown in Chart 3; 95% of the values ranged from 0.1 to 25 nmol/g tissue. A few samples showed tissue concentrations higher than the peripheral plasma levels. In Group 2 (premenopausal cancer patients) and in the hypertrophic tissue in Group 5, most tissues showed concentrations below the corresponding plasma levels.

Comparison of Androgen Concentrations in the Different Groups. In premenopausal women, the concentration of DHEA in the cytosol of normal tissue (Group 1) tended to be higher (p < 0.10) than in malignant tissue (Group 2). No significant differences were found in nuclear and tissue concentration of DHEA. With respect to ADIOL, no significant differences could be demonstrated. In the tissues of premenopausal women in Group 5, the concentration of DHEA-S in the cytosol is significantly higher than in premenopausal malignant tissue of Group 2 (P < 0.02). Normal tissues were available from only 6 postmenopausal patients; no significant differences in DHEA, ADIOL, and DHEA-S concentrations were found in postmenopausal Groups 3 and 4.

No differences were found in the concentration of DHEA between pre- and postmenopausal malignant tissue, although median concentrations in cytosol, nuclear fraction, and tissue are higher in the premenopausal group. The concentrations of ADIOL tended to be higher (P < 0.10) in the cytosol of the premenopausal patients; in the nuclear fraction and in the total tissue, the concentrations are significantly higher (P < 0.05). The concentration of DHEA-S tended to be higher in the premeno-
The concentration of DHEA in normal tissue is significantly higher in the nuclear fraction and tissues of premenopausal patients (Group 1) compared with postmenopausal patients (Group 3) ($P < 0.02$ and $< 0.05$, respectively). The concentration of ADIOL tended to be lower ($P < 0.10$) in the cytosol, nuclear fractions, and tissue of the postmenopausal group compared with the premenopausal group (Group 3 versus Group 1). DHEA-S in the cytosol is significantly lower in the postmenopausal tissues ($P < 0.05$).

Group 5 contains 3 subgroups of tissues from premenopausal patients, with nonmalignant histology (hypertrophic tissue, fibrocystic tissue, and benign tumor tissue). The concentrations of DHEA and ADIOL in the hypertrophic tissues were comparable to those found in normal postmenopausal tissues and were lower than in normal premenopausal tissue. No difference in DHEA-S levels in the cytosol was found among the 3 subgroups.

The ADIOL, DHEA, and DHEA-S concentrations in the cytosol and nuclear fractions and in the tissue of fibrocystic and benign tumors were comparable to those in normal premenopausal tissue. Concerning the subcellular distribution, the concentrations of DHEA and ADIOL in the hypertrophic tissues were comparable to those found in normal postmenopausal tissues and were lower than in normal premenopausal tissue. No difference in DHEA-S levels in the cytosol was found among the 3 subgroups.

Androgen Concentrations in Relation to Age. With increasing age, a decrease in the production rate of androgens has been reported which is reflected in decreasing plasma levels.

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Chart 2. Median values of ADIOL levels in the cytosol and nuclear fractions and in tissue in the 5 groups of women. The range of plasma levels of healthy pre- and postmenopausal volunteers is given. Tissue levels of Groups 3 and 4 should be compared with the plasma levels of postmenopausal women, while tissue levels of the other groups should be compared with those of premenopausal women. Group 5 represents 2 different subgroups: hypertrophic tissue (•); benign tumor and fibrocystic tissue (○).

Chart 3. DHEA-S levels in the cytosol in the 5 groups of women. Groups 3 and 4 represent postmenopausal women. Groups 2 and 4 represent malignant breast tumor tissues of pre- and postmenopausal women, respectively. Group 5 represents 2 different subgroups: hypertrophic tissue (•); benign tumor and fibrocystic tissue (○).
Therefore, the correlation of the tissue concentration of DHEA, ADIOL, and concentrations of DHEA-S in the cytosol with age was calculated separately for healthy subjects and patients (only correlations with \( r > 0.50 \) have been considered). In Groups 3 and 4, a negative correlation for DHEA-S was found which was significant: Group 3, \( r = -0.89, P = 0.01, n = 6 \); Group 4, \( r = 0.82, P = 0.001, n = 29 \). In the same groups, negative correlations were found for DHEA, but the correlation was significant only for Group 4. \( r = -0.51, P = 0.002, n = 32 \). No correlation was found for ADIOL. Taking all patients together, low but significant correlations were found for DHEA \((r = -0.33, P = 0.001, n = 110)\), ADIOL \((r = -0.33, P = 0.001, n = 91)\), and DHEA-S \((r = -0.46, P = 0.001, n = 109)\).

Since DHEA-S can act as a precursor for DHEA and ADIOL, we compared the correlation between DHEA-S and DHEA and between DHEA and ADIOL. Only groups with sufficient pairs (Groups 1, 2, 4, and 5) have been considered. The correlation between DHEA-S and DHEA was highly significant in all these groups \( (P < 0.005) \). For all patients, the correlation was \( r = 0.75 \) \( (P < 0.001, n = 105) \). The correlation between DHEA and ADIOL was also highly significant for all groups; collectively, the correlation was \( r = 0.88 \) \( (P < 0.001, n = 91) \).

**ADIOL:Estradiol Ratio.** In view of our hypothesis on the role of ADIOL in the modulation of estradiol action, data on the concentrations of estradiol in the same samples, which have been published previously \((18)\), was used to calculate the ADIOL:estradiol ratio for each tissue sample (Chart 4). In the cytosol, a significant difference was found between normal and malignant tissues of premenopausal women (Group 1 versus Group 2, \( P < 0.05 \)). This difference was not observed in postmenopausal women (Group 3 versus Group 4). In the nuclear fraction, markedly high ratios are found in all groups, 95% of the ratios range from <1 to 40 in cytosol and from <1 to about 100 in the nuclear fraction and in tissues. No differences in the ratios were found between normal and malignant breast tissues of patients of the same menopausal status.

**DISCUSSION**

Recently, we have published a reproducible and reliable method for the estimation of steroid hormones in human tissue \((16)\). After extraction and purification of the samples by column chromatography, the steroids were estimated by specific radiomunoassays. Because DHEA-S has a high concentration (in nmol) in comparison to the other androgens (in pmol), DHEA-S was measured directly in the cytosol.

Our data on the tissue concentrations of DHEA and ADIOL agree with those reported by Maynard and Griffiths \((10)\), who measured these steroids by gas chromatography-mass spectrometry. Tissue concentrations of DHEA are similar to those reported by Adams et al. \((2)\), although Adams et al. reported higher ADIOL levels using the same antibody. The direct measurements of ADIOL without chromatographic purification may explain the difference in ADIOL concentrations. Data on the concentration of DHEA-S in human breast tissues are not available in the literature.

The tissue-plasma gradient was most pronounced for DHEA; because we measured DHEA-S only in the cytosol, we are unable...
to calculate the final tissue-plasma gradient for this steroid. However, the low concentrations of DHEA-S in the cytosol as compared to peripheral plasma levels make it unlikely that there is a distinct tissue-plasma gradient. The absence of this gradient can be explained by its relatively strong binding to albumin (19) and its higher polarity compared to DHEA and ADIOL, which may result in a slower entry of DHEA-S into the cell. The highly significant correlation between the 3 androgens measured suggests an active intracellular metabolism of DHEA-S to DHEA and ADIOL.

Data clearly show that the concentration of DHEA-S in non-malignant tissues of premenopausal women is significantly higher than in malignant tissues. The higher concentrations of DHEA-S are reflected only in higher concentrations of DHEA in the cytosol of nonmalignant tissues.

Significant differences were found in the total tissue concentration of ADIOL between malignant and nonmalignant tissues of premenopausal women. No difference in androgen concentrations were found between malignant and nonmalignant tissues of postmenopausal women. Why the concentrations of DHEA and, to a lesser extent, of ADIOL and DHEA-S in hypertrophic tissues are relatively low and comparable with concentrations in tumor tissues of postmenopausal women is unclear.

As a group, a low but significant negative correlation was found between the concentration of the 3 androgens in the tissue and the age of the patients. This can explain the differences between pre- and postmenopausal women. In contrast to our results with estrone and estradiol in the same tissues (18), the concentration of ADIOL, but especially of DHEA, was generally higher in the nuclear fraction. Maynard et al. (19), however, reported similar concentrations in the cytosol and nuclear fractions. Recently, Adams et al. (2) reported the concentration of DHEA and ADIOL in nuclei, purified from only one pool of 4 different tumors. They reported that the concentration of ADIOL, and especially of DHEA, was much higher in the cytosol than in the nuclear fraction. Binding of DHEA and ADIOL to cellular material other than nuclei may be the cause of the conflicting results obtained by Adams et al., Maynard et al., and us. The mechanism that contributes to a high nuclear:cytosol ratio of DHEA and ADIOL is unknown. It is possible that specific binding proteins are involved. Such a specific binding protein for DHEA and ADIOL, present only in the nucleus of rat vagina tissue, has been demonstrated (13). These findings, however, could not be reproduced in our laboratory. Barrack and Coffey (3) have reported recently the presence of specific binding proteins in the nuclear matrix of human material.

We reported a significantly higher concentrations of estradiol in malignant tissue. These concentrations were measured in the same cytosols and nuclear fractions as used in the present study (18). In the present study, no differences were found in the ADIOL:estradiol ratio for the total tissues in normal and malignant breast tissue of patients of the same menopausal status. On the other hand, we found that the ADIOL:estradiol ratio in the nuclear fraction of malignant tissue is lower than in the nonmalignant tissues. This lower ratio was mainly caused by higher concentrations of estradiol, rather than by lower concentrations of ADIOL. This suggests that our hypothesis of the antagonizing action of ADIOL at equal concentrations of estradiol in women with and without breast cancer is not supported by our present data. After initiation of our study, analysis of the plasma levels of ADIOL in our laboratory in patients with breast cancer or with a benign mammary disease and in women at high risk for mammary cancer showed no significant difference. In addition, van Doorn et al. (14) found that ADIOL has potential estrogenic activity in the immature rat uterus, since it binds to the estradiol receptor, translocates the receptor into the nucleus, and induces specific estrogenic effects such as the induction of the progesterone receptor (14). These findings also do not support our original hypothesis of the inhibitory role of ADIOL. The higher concentrations of DHEA and ADIOL in normal tissues than in malignant ones do not support the suggestion of Adams et al. (1) that higher concentrations of free DHEA and ADIOL should result in higher concentrations of unsulfurylated estradiol because of the inhibitory action of both androgens on the estrogen-sulfurylating enzyme in vitro. Moreover, our data also do not support a more recent suggestion of Adams et al. (2) that DHEA and DHEA-S could provoke a “carcinogenic” stimulus via their metabolite ADIOL. During the preparation of this paper, interesting observations were published on a possible role of adrenal androgens as regulators of estrogenic activity. Bonney et al. (4) found that both ADIOL and DHEA and DHEA-S are inhibitors of 17β-OHSD in endometrial tissue. These findings imply that ADIOL, DHEA, and DHEA-S influence the activity of 17β-OHSD at cellular levels, resulting in a decreased conversion of estradiol to estrone and therefore leading to higher concentrations of estradiol in the tissue. Because we did not find higher androgen levels in the malignant tissue, the higher estradiol levels in these tissues cannot be explained by inhibition of 17β-OHSD by either ADIOL, DHEA, or DHEA-S.

At the moment, it is impossible for us to make a unifying hypothesis which is consistent with all the data from our work and from the literature.

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