ties after being treated with substances secreted by EAC, probably due to diminished production of the suppressor factor.

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Androstenedione Conversion in Human Peripheral Blood Lymphocytes

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The ability of human peripheral blood lymphocytes to convert the androgenic steroid metabolite androstenedione diminishes in women over 45 years old or during menopause. In patients with breast cancer in menopause the ability of lymphocytes to convert androstenedione is enhanced compared with the control. The intensity of conversion in circulating lymphocytes correlates with the blood concentration of sex steroids.

Key Words: *androstenedione; lymphocytes; peripheral blood*

Lymphocytes have surface and cytosol receptors for various hormones [9]. However, these cells have recently been regarded not only as sites of hormonal action but also as sites of hormone synthesis and metabolism. In fact, lymphocytes can produce a number of peptide hormones or hormone-like factors [4] and metabolize one of the most active human glucocorticoids - cortisol [8]. Previously [1] we speculated that lymphocytes may be a site of extragonadal synthesis of estrogens: a process consisting of aromatization of androgenic precursors (notably androstenedione (A) and/or testosterone) and being an intrinsic property primarily of fat and muscle tissues, i.e., tissues which are peripheral in relation to the gonads and adrenals [12]. This assumption was based on observations of $^3H_20$ formation during incubation of ^{3}H-1β-A with human lymphocytes [1]. Due to its stoichiometric peculiarities (theoretically, 1 M estrogens should be formed per M $^3H_20$), this reaction has been used for the evaluation of aromatous activity since the mid-1970s [13]. However, later

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experiments with endometrium, prostate, liver [6,10,11], and lymphocytes [3] have shown that production of estrogens upon conversion of A evaluated by the formation of heavy water frequently does not occur. Therefore, this reaction was termed by us pseudoaromatization [3], and although the study of the products of this reaction is not complete, the conversion of A in these tissues and cells with the formation of new (other than estrogens) biologically active steroids is a definite fact [6,10]. Taking into account the effect of sex hormones on the immunity and growth of some tissues, the investigation of this reaction in human lymphocytes (including those obtained from cancer patients) seems to be of interest.

Our objective was to study the conversion of A in lymphocytes and to compare it with some parameters of hormonal-metabolic status in healthy women and women with breast cancer (BC).

MATERIALS AND METHODS

The study included 74 women: 38 were healthy and 36 suffered from BC. The age of the healthy women ranged from 20 to 66 years and the age of BC patients ranged from 25 to 72 years. Twelve healthy women and 25 patients were in a more than 1-year-long menopause. Most of the patients had II-III stage BC. The patients were investigated at their first request without any preceding therapy. Lymphocytes were isolated on a Verografin-Ficoll gradient from blood obtained after a 10-12-h fast [5]. The conversion of A was assessed as previously: in a radioisotope assay by the formation of heavy water from ^{3}H-1β-A [3,13]; the protein content was determined after Lowry. Body weight, height, waist, and thigh measurements were recorded. The serum contents of glucose, total low and very low density lipoproteins, triglycerides, total cholesterol, cholesterol of high density lipoprotein, insulin, testosterone, estrogens, and progesterone were measured using standard enzyme colorimetry and radioimmune assay [2]. Statistical analysis included calculation of the mean, standard error of the mean, and assessment of distributions. Since the distributions were often not normal, the χ^2 and Wilcoxon-Mann-Whitney tests and Spearman's rank correlations were employed.

RESULTS

The major parameters characterizing the specific features of A conversion in peripheral blood lymphocytes in healthy subjects and BC patients are summarized in Table 1. Although there was no difference between these groups in the mean data, further analysis showed that in BC patients in menopause or in patients over 45 the intensity of A conversion in lymphocytes is significantly higher than in the corresponding groups of healthy women.

We failed to establish any relationship between the conversion of A and body weight, its excess relative to the "ideal" value, topography of fat deposits, blood sugar, α-cholesterol, triglycerides, lipoprotein, and insulin. A positive correlation between the intensity of A conversion and cholesterolemia was established in healthy climacteric women. On the other hand, both in healthy women and in BC patients in menopause there was a positive correlation between the level of A conversion in lymphocytes and the blood concentration of estrogens, with a clear tendency toward negative correlation with the blood concentration of progesterone. The tendency toward a positive correlation with the testosterone level was observed only in BC patients who were still menstruating (the correlation coefficients are given in Table 2).

Thus, our results confirm the ability of lymphocytes to convert A [1,3]. In addition, this is the first piece of evidence demonstrating a decline in this ability in women over 45 or, to be more precise, during menopause. A much lesser decrease in the intensity of A conversion during menopause occurs in patients with BC, and during this period A conversion is significantly higher than in healthy women. In contrast to their immunocompetent properties (the metabolic depression phenomenon [2]), the ability of lymphocytes to metabolize A is only to a small degree dependent on lipocarbohydrate metabolism. At the same time, from the viewpoint of the hormonal regulation of A conversion in circulating lymphocytes it is important that during menopause the intensity of this reaction increases at a decreased level of progest-

TABLE 1. Conversion of A in Peripheral Blood Lymphocytes ($M \pm m$)

Group	Conversion of A, fM/mg protein/hour	
	healthy women	BC patients
All examinees	11.5±1.3 (38)	11.0±1.3 (36)
Reproductive period	14.3±1.6 (26)	13.6±2.1 (11)
Menopause	5.4±0.5** (12)	9.9±1.6* (25)
⩽45 years	14.3±1.6 (26)	13.4±2.6 (9)
⩽45 years	5.4±0.5*** (12)	10.2±1.5* (27)

Note. The number of observations is given in parentheses. Asterisks indicate a statistically significant difference ($p<0.05$) with healthy women (one asterisk), with healthy women in the reproductive period (two asterisks), and with women under 45 (three asterisks).

TABLE 2. Coefficients of Rank Correlation between the Intensity of A Conversion in Lymphocytes and Some Parameters of Hormonal – Metabolic Status

Group	Status	Coefficient of correlation with						
		body weight	excess body weight	insulin	cholesterol	estrogens	progesterone	testosterone
Healthy subjects	Reproductive period	−0.06	−0.10	0.24	−0.18	−0.30	−0.02	0.04
	Menopause	−0.03	0	−0.03	0.61*	0.66[1]	−0.60	−0.07
Patients with BC	Reproductive period	−0.20	0.06	−0.24	−0.35	0.14	−0.11	0.63
	Menopause	0.15	0.23	−0.31	0.12	0.77**	−0.63	0.15

Note. In more advanced menopause (≥2 years) this correlation is more pronounced and statistically significant ($r=0.76$, $p<0.05$). Asterisks indicate the significance of differences at $p<0.05$ (one asterisk) and $p<0.01$ (two asterisks).

erone and particularly in the case of pronounced estrogenemia.

In an attempt to answer the question whether the intensification of A conversion in lymphocytes in postmenopausal patients with BC has any biological significance, we should first consider the biochemical principles of this reaction. Concerning the pseudoaromatization reactions in the prostate, Brodie *et al.* [6] suggested that A is hydroxylated at the first C atom. In our experiments with A labeled not only at position 1, but also at positions 2, 6, and 7 the formation of heavy water during conversion of A in lymphocytes increased considerably [3]. Similar results were obtained in the studies of pseudoaromatization in other tissues [6,10]. These findings suggests that this reaction may proceed not only at the first C atom in the androgen molecule.

It was also demonstrated that in the prostate the reaction of pseudoaromatization proceeds in several steps, the first being a 5α-reductase reaction which is followed by a putative hydroxylation [6]. Thus, the possibility cannot be excluded that although estrogens are not formed during A conversion in lymphocytes [3], other biologically active compounds may be formed as a result of this reaction, for example 5α-dihydrotestosterone, which has an immunodepressive activity [7], or other steroids, including those modulating the effect of estrogen.

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