Abstract

Normal young and postmenopausal women were placed into groups according to the ratio of the estrogens in their urine. Women whose ratio was >1.3 if young and >3.2 if postmenopausal were compared to women whose ratio was <0.7 and <2.1 for young and postmenopausal, respectively. Between the respective high- and low-ratio groups, there were no significant differences for circulating levels of estriol, metabolic clearance rates of estradiol, or blood production rates of estriol, estrone, or estradiol.

Women who had had breast cancer were compared to a group of normal controls and were also found to have similar blood production rates for estriol, estrone, and estradiol.

The ratios of the blood production rates of estriol to estrone and estradiol were similar for the high and low groups for young and postmenopausal women and also between the breast cancer women and their controls.

It appears, therefore, that the difference in urinary estrogen ratios is primarily due to different pathways of metabolism of the free circulating estrogens and not to differences in the production rates of the estrogens. Estriol is produced at only 10% the rate of estrone and estradiol.

Introduction

The role of estriol in the epidemiology of breast cancer has been the subject of controversy for a number of years. Following the studies of Hisaw et al. (12) and Huggins and Jensen (13), the 3 classical estrogens, estradiol, estrone, and estriol, were characterized as having differing biological activities. Estradiol and estrone were considered to be true estrogens stimulating uterine growth in similar fashion but differing somewhat in potency. In contrast, estriol was considered to be an impeded estrogen since it appeared to interfere with the action of estrone and estradiol on uterine growth, although by itself it possessed some uterine growth-stimulating ability. Subsequently, Lemon et al. (16, 17) reported that the ratio of estriol conjugates to estradiol plus estrone conjugates in the urine (the urinary ratio) could identify people at high and low risk for breast cancer. From these studies evolved the concept that estriol might have a protective role in breast cancer and would be present in larger amounts in women at low risk and in smaller amounts in women at high risk for breast cancer.

MacMahon et al. (21) subsequently reported that Oriental populations who were at low risk for breast cancer had higher ratios of estriol, estrone, and estradiol in the urine than did Caucasian women who were at higher risk for breast cancer.

The studies of Lemon et al. (16, 17) and of MacMahon et al. (21) were carried out according to the method of Brown (6) which measures the amounts of estrone, estradiol, and estriol conjugates in the urine. These urinary estrogen conjugates are primarily glucuronides although there are sulfates as well (6, 8, 24). However, as measured in urine, the conjugates of the 3 estrogens constitute only a small portion of the total amount of the parent estrogens that are produced in the body (8, 9). In addition, circulating estrone and estradiol can both contribute similarly to all 3 estrogen conjugates in the urine (5). The parent unconjugated, unmetabolized (free) estrogens are presumably biologically active.

The ratios of conjugated estriol to estrone plus estradiol conjugates could vary because of a difference in the production rates of the free estrogens (Chart 1) which are then metabolized along similar pathways leading to the excretion of the conjugates in a ratio that would resemble that found for the free estrogens in the blood. However, it is also possible that the urinary ratios could differ because of differences, not in secretion, but rather in metabolism (Chart 2) and that the free estrogens would be secreted in relatively equal amounts irrespective of the urinary ratios. The differing ratios in the urine would reflect merely a difference in pathways of metabolism. It has been shown previously that urinary estriol arises in part from secreted estradiol and estrone (5, 8, 24) although it has been suggested by Barlow and Logan (4) and Eren et al. (9) that in the luteal phase of the cycle there was secretion of estriol itself. However, there have been no studies on the relationship between urinary ratios and the production rates of these estrogens throughout the cycle. Therefore, the studies to be reported here were carried out in an attempt to see whether women with differing urinary estrogen ratios had different blood production rates of the estrogens.

Methods

Normal, healthy women collected 24-hr urine specimens during the follicular phase, Days 5 to 7, and the luteal phase, Days 20 to 22, of their cycle. The estrone, estradiol, and estriol levels in these urines were then measured by the method of Brown (6). Women whose ratios were greater...
than 1.3 or less than 0.7 were used for subsequent studies. Normal, healthy postmenopausal women collected 24-hr specimens and on the basis of their urinary ratios were divided into 2 groups, those whose ratios were greater than 3.2 and those whose ratios were less than 2.1. An additional group studied without regard to their urinary ratios consisted of women who had had breast cancer but had no clinical evidence of the disease at the time of the study.

For all women measurements were made of the concentrations and the metabolic clearance rates of the estrogens, as described previously (11, 19, 22, 23). Blood samples were drawn from the women for analyses of estrone, estradiol, and estriol by radioimmunoassay (20, 23), and then concentrations and the metabolic clearance rates of the estrogens, and the excretion of estrogen conjugates in the urine based on similar estrogen secretion rates but variations in metabolic pathways. $E_1$, estrone; $E_2$, estradiol; $E_3$, estriol; $\bullet$, other estrogen conjugates.

Results

Concentration of Estriol. Plasma concentrations of estriol and estradiol in normal women were measured throughout the cycle as shown in Chart 3. The levels of estriol were found to be generally about 10% of the estradiol concentration. In addition, there were increases in plasma estriol concentrations that appeared to be associated with those of estradiol, and there were also increases that were independent of estradiol, perhaps suggesting the secretion of small amounts of estriol directly from the ovary (4, 9). In a larger group of women studied at 2 times of the cycle only, there was a rise in estriol during the luteal phase of the cycle, and for Days 20 to 22 the mean ± S.E. value (values given are mean ± S.E. unless stated otherwise) of 10 ± 1 pg/ml was significantly ($p < 0.01$) higher than the mean value of 8 ± 1 pg/ml for Days 5 to 7 of the cycle. When the amounts of estriol present in the plasma in the low-ratio as opposed to the high-ratio women were compared (Chart 4), there were no differences ($p > 0.1$) between the groups (19). The values were similar in the follicular phases for the 2 groups and in the luteal phase for the 2 groups. The mean values for the concentrations in postmenopausal women were similar ($p > 0.1$) in the high (5 ± 2 pg/ml)- and low (7 ± 2 pg/ml)-ratio groups and in those women with breast cancer (7 ± 2 pg/ml), as compared to controls (6 ± 1 pg/ml) (22).

Metabolic Clearance Rates. The metabolic clearance rates for the 2 groups were not significantly different ($p > 0.1$) either in the follicular phase or in the luteal phase (Chart 5). The mean metabolic clearance rates of estriol were 1270 ± 90 and 1400 ± 90 liters/day/sq m in the follicular phase and 1320 ± 100 and 1440 ± 100 liters/day/sq m in the luteal phase for the high- and low-ratio groups, respectively (19). These values suggest that there is little important globulin binding of estriol (2, 3), since the values are similar to those for androstenedione and estrone which are also not bound to plasma globulins (2, 3). The mean metabolic clearance rates were not different ($p > 0.1$) between the postmenopausal women at 1090 ± 20 liters/day/sq m and 1160 ± 100 liters/day/sq m for high- and low-ratio groups or between the breast cancer and control groups, at 950 ± 70 and 1150 ± 90 liters/day/sq m, respectively (22).

Blood Production Rates. The production rates of estriol are similar in the high-ratio as opposed to the low-ratio group in the follicular and the luteal phases of the cycle (Chart 6). The mean production rates of estriol in the luteal
versus 108 and 196 /¿g/day) are similar for the high- and versus 67 and 156 jug/day) and estradiol (100 and 211 Therefore, it would appear that the differences in the uri
differences in the pathways of metabolism (Chart 2). There-

to demonstrate a difference in the circulating levels of es

trogens in the groups of women studied, we were unable

0.10 ± 0.01 in the follicular and 0.07 ± 0.01 versus 0.07 ± 0.02 in the luteal phase for the high-ratio and low-ratio groups, respectively. The ratios of the blood production rates in the breast cancer and control groups are also not significantly different (p > 0.1) (Chart 9). The ratios are 0.16 ± 0.04 for the cancer group and 0.23 ± 0.06 for the control group.

The Ratios of Blood Production Rates. As shown in Chart 8, the ratios of the blood production rates for the estrogens are similar in the high- and low-urinary-ratio groups. The mean ratios in the blood for estriol compared to the sum of estrone and estradiol were 0.09 ± 0.01 versus 0.10 ± 0.01 in the follicular and 0.07 ± 0.01 versus 0.07 ± 0.02 in the luteal phase for the high-ratio and low-ratio groups, respectively. The ratios of the blood production rates in the breast cancer and control groups are also not significantly different (p > 0.1) (Chart 9). The ratios are 0.16 ± 0.04 for the cancer group and 0.23 ± 0.06 for the control group.

Discussion

Despite a wide variation in the urinary ratios for the estrogens in the groups of women studied, we were unable to demonstrate a difference in the circulating levels of es-
triol, in the metabolic clearance rates of estriol, and in the blood production rates of estrone, estradiol, and estriol. Therefore, it would appear that the differences in the uri-
naries between the 2 groups are a reflection not of vari-
ations in the production rates of the estrogens but rather of differences in the pathways of metabolism (Chart 2). There-

phase are 22 ± 2 and 25 ± 2 µg/day for high- and low-ratio
groups and are not significantly different (p > 0.1) from the respective follicular-phase values of 17 ± 2 and 19 ± 2 µg/ day. The mean production rates of estrone (85 and 171 versus 67 and 156 µg/day) and estradiol (100 and 211 versus 108 and 196 µg/day) are similar for the high-

and low-ratio groups (although for each group the values are higher in the luteal phase of the cycle). The postmenopau-
sal women also had similar production rates of estrone, estradiol, and estriol (Chart 7) despite differences in urinary ratios. The blood production rates of the estrogens were not different (p > 0.1) for the breast cancer group and for the control group of women.

Chart 5. Metabolic clearance rates of estriol in normal women grouped according to time of cycle and urinary ratio.

Chart 6. Blood production rates of estrogens in the follicular and luteal phases of the cycle for high and low urinary ratios. E₁, estrone; E₂, estradiol; E₃, estriol.

Chart 7. Blood production rates of estrogens in normal postmenopausal women with high and low urinary ratios and in postmenopausal women with breast cancer. E₁, estrone; E₂, estradiol; E₃, estriol.

Chart 8. Ratio of the blood production rates (E₁/E₂ + E₃, in which E₁ is estrone, E₂ is estradiol, and E₃ is estriol) of the estrogens in normal women grouped according to time of cycle and the urinary ratio.

Chart 9. Ratio of the blood production rates (E₁/E₂ + E₃, in which E₁ is estrone, E₂ is estradiol, and E₃ is estriol) of the estrogens in postmenopausal women with breast cancer and controls.

Therefore, both groups of women have similar amounts of biologically active estrogens entering the blood, but in the tissues there is a subsequent change or diversion in the metabolic pathways to divide women into those with high and those with low urinary ratios of the 3 classical estrogens. The metabolic clearance rate that we measure is defined as "that volume of blood cleared irreversibly of a steroid in unit time" (2, 3) and it is independent of the pathways involved in steroid metabolism in peripheral tissues. Thus, variations can occur in the later pathways of metabolism, after the steroid has irreversibly left the blood without affecting the metabolic clearance rates.

MacMahon et al. (21) and others (1), including ourselves (19), have noted that the difference in the urinary ratios actually is more a reflection of differences in the excretion rate of the conjugates of estrone and estradiol than of differences in the excretion rate of estriol. It would appear, therefore, that these groups of women produce similar amounts of estrone, estradiol, and estriol. However, in the high-ratio group a large fraction of the estrone and estradiol is metabolized along pathways leading to a conjugate which is not measured as a classical estrogen and that in the low group the fraction that is transformed into an unmeasured conjugate(s) is much less. One of the major groups of estrogen metabolites that would not be measured by the procedure of Brown (6) would be the catechol estrogens as reported by Fishman (10). It is possible that there is an increase in the formation of catechol estrogens in women with high estrogen ratios as opposed to the women with low estrogen ratios. Further studies on this aspect would be of interest.

We believe that the urinary ratios of estrogen, while they
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may be a marker for women at high or low risk for breast cancer, do not appear to reflect differences in the amounts of the biologically active estrogens entering the blood; therefore estriol probably plays only a minor role in the actual etiology of the disease. This view is further strengthened, we believe, by a consideration of the amounts of estrogens entering the blood. The amount of estriol entering the blood is only 10% or less of the amounts of the other estrogens entering the blood. As shown by Clarke et al. (7) and others (15, 18), estriol appears to be a weak estrogen and probably not an impeded estrogen as originally suggested by the studies of Huggins and Jensen (13). Therefore estriol, a weak estrogen that enters the blood at levels of only one-tenth that of the more potent estrogens, would probably play only a minor role in the overall biological activity of the estrogens at the level of the target tissues.

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References

Relationship between Urine and Plasma Estrogen Ratios

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