Methods and Results of Aromatization Studies in Vivo

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Abstract

In order to characterize aromatization in vivo, an androgen and an estrogen, labeled with different radioactive isotopes, are administered simultaneously, either as a pulse alone or as a pulse followed by a constant infusion of the same radiolabeled steroids. Subsequently, blood samples are obtained at varying time intervals and analyzed for the $^{3}$H/$^{14}$C ratio in nonmetabolized unconjugated estrogen, and/or all urine is collected for 96 hr and analyzed for the $^{3}$H/$^{14}$C ratio in an estrogen conjugate. The fraction of androgen (An) entering the blood which is converted to and measured as a specific estrogen (Es) in the blood $\left(\frac{\text{An}}{\text{Es}}\right)_B$ is calculated from

$$\left(\frac{\text{An}}{\text{Es}}\right)_B = \frac{\left(\frac{\text{H-Es}}{\text{H-An}}\right)_M}{\left(\frac{\text{H-An}}{\text{H-Es}}\right)_M}$$

where $\left(\frac{\text{H-Es}}{\text{H-An}}\right)_M$ is the ratio of radioactivities in the specific estrogen conjugate in the body and measured as a specific estrogen conjugate in the urine $\left(\frac{\text{H-Es}}{\text{H-An}}\right)_U$ is calculated from

$$\left(\frac{\text{H-Es}}{\text{H-An}}\right)_U = \frac{\left(\frac{\text{H-Es}}{\text{H-An}}\right)_{\text{ini}}}{\left(\frac{\text{H-An}}{\text{H-Es}}\right)_{\text{ini}}}$$

where $\left(\frac{\text{H-An}}{\text{H-Es}}\right)_{\text{ini}}$ is the ratio of radioactivities in the specific estrogen conjugate. Irrespective of the method used, the aromatization of androstenedione is greater than the aromatization of testosterone; aromatization in normal men is greater than that in normal young women; aromatization in postmenopausal women is greater than that in premenopausal women.

Aromatization occurs in many tissues but especially in adipose tissue and muscle, and to a lesser extent in brain, kidney, and skin. The liver appears to be a relatively minor site for aromatization in normal subjects.

In certain diseases, e.g., hepatic cirrhosis and obesity, aromatization rates are increased compared to those rates in normal individuals.

In men and postmenopausal women, peripheral aromatization of androgen is a major source of circulating estrogens. The peripheral aromatization of an androgen does not appear to be influenced by luteinizing hormone, follicle-stimulating hormone or adrenocorticotropic hormone or by substrate or product concentration. Blood flow through peripheral tissues may play a major role in control of this physiologically important reaction.

Although there were indications from the early work of Leach et al. (10) that androgens could be aromatized to estrogens by peripheral extraglandular tissues, aromatization by these tissues was not established until the work of MacDonald et al. (18) and Longcope et al. (15). These workers administered radiolabeled androgens to normal subjects and after extensive purification procedures found radiolabeled estrogens in the urine and blood. Studies since then have been directed at determining the importance of peripheral aromatization as a source of estrogens, the tissues involved in the reaction, disease states which alter the rate of aromatization, and the controlling mechanisms of the reaction. These studies have been carried out using analysis in either blood or urine, and I will review briefly the 2 techniques involved and present and discuss the results.

Methods

Analysis in Blood (2, 6, 15, 16). The subjects were given an i.v. priming dose of an $^3$H-labeled androgen and a $^{14}$C-labeled estrogen in 0.9% NaCl solution followed by a constant i.v. infusion for 2 to 4 hr of the same radiolabeled steroids. During the course of the infusion, blood samples were obtained from a vein in the arm opposite to the infusion. In certain experiments, blood samples were obtained from multiple veins and an artery in order to establish tissue aromatization rates.

The samples were analyzed for radioactivity as specific androgens and estrogens by standard methods involving solvent extraction followed by extensive purification procedures consisting of multiple chromatographies and derivative formation until radiochemical purity was achieved. The radioactivity in the samples was then measured by liquid scintillation spectrometry, and the results were corrected for losses through the procedure.

The amount of radioactivity administered as $^3$H-labeled androgen which enters the blood as the specific estrogen during the infusion is calculated as

$$^3\text{H-Es} \times \text{MCRE}_{\text{Es}}$$

where $^3\text{H-Es}$ is the concentration of $^3$H in the specific estrogen and MCRE$_{\text{Es}}$ is the metabolic clearance rate of the specific estrogen. The fraction of $^3$H-labeled androgen administered which is aromatized to the specific estrogen $\left(\frac{\text{MCRE}_{\text{Es}}}{\text{MCRE}_{\text{An}}}ight)$ is also calculated as

$$\left(\frac{^3\text{H-Es}}{^3\text{H-An}}\right)_{\text{ini}} \times \text{MCRE}_{\text{Es}}$$

where $^3\text{H-An}$ is the rate of infusion of $^3$H-labeled androgen. This can also be expressed as

$$\left(\frac{^3\text{H-Es}}{^3\text{H-An}}\right)_{\text{ini}} = \left(\frac{^3\text{H-Es}}{^3\text{H-An}}\right)_{\text{ini}} \times \text{MCRE}_{\text{Es}}$$

where $\text{MCRE}_{\text{Es}}$ is the $^3$H/$^{14}$C ratio of radioactivity administered as the specific estrogen in the blood and $\left(\frac{^3\text{H-An}}{^3\text{H-Es}}\right)_{\text{ini}}$ is the $^3$H/$^{14}$C ratio of administered radioactivity, both androgen and estrogen.

This method gives the fractional rate of aromatization of androgen to estrogen where the estrogen must enter the blood pool of circulating unmetabolized estrogen, i.e., Chart 1, Step 2. Inherent in the technique is the establishment of radiochemical purity as noted and also the achievement of an isotopic steady state for the androgen and estrogen radioactivity in the blood during the infusion.
With this type of analysis, the production rates of androgens and estrogens are determined at the same time as the fractional aromatization rates are calculated. Therefore, the mass of androgen aromatized to estrogen and the importance of this aromatization can be determined.

Analysis in Urine (17, 18). The subjects received radiolabeled androgens and estrogens either as a pulse or as a priming dose, followed by a constant infusion of the same radiolabeled steroids. Following the administration of radioactivity, all urine was collected for 3 to 5 days and pooled. An aliquot equal to 50 to 100% of the urine was extracted with an organic solvent to remove any free steroid, and the urine was then subjected to β-glucuronidase hydrolysis. After hydrolysis, the urine was extracted with organic solvent, and the unconjugated steroids were purified by multiple chromatographies and derivative formation. Because the amounts of radioactivity involved are larger than in the blood specimens, most procedures have included crystallization to constant specific activity for final purification.

The fractional rate of aromatization was calculated as

\[
\frac{[p]^{\text{BM,Es}}_{\text{EE}}}{[p]^{\text{BM,An}}_{\text{EE}}} = \left( \frac{^{3}H-\text{Es} / ^{14}C-\text{Es}}{^{3}H-\text{An} / ^{14}C-\text{Es}} \right)^{\text{BM}}
\]

where \( ^{3}H-\text{Es} / ^{14}C-\text{Es} \) was the ratio of radioactivity as \(^3\)H to \(^{14}\)C in the urinary estrogen conjugate.

As shown in Chart 1, \([p]^{\text{BM,Es}}_{\text{EE}}\) is a measure of Step 2 which represents all the unconjugated estrogen entering the blood after its aromatization from androgen in peripheral tissues. However, \([p]^{\text{BM,Es}}_{\text{EE}}\) is a measure of Step 1 which represents all the estrogen formed from androgen in the tissues, whether it enters the blood (Step 2) or is conjugated to \(E_{\text{Conj}}\) (Step 3) before it enters the blood. Therefore,

\[
[p]^{\text{BM,Es}}_{\text{EE}} = [p]^{\text{BM,Es}}_{\text{EE}} + 3
\]

If Step 3 is nonexistent, then \([p]^{\text{BM,Es}}_{\text{EE}}\) will be equal to \([p]^{\text{BM,Es}}_{\text{EE}}\). However, if Step 3 is present, then \([p]^{\text{BM,Es}}_{\text{EE}}\) will be smaller than \([p]^{\text{BM,Es}}_{\text{EE}}\) by an amount equal to Step 3.

An additional cause for a discrepancy between \([p]^{\text{BM,Es}}_{\text{EE}}\) and \([p]^{\text{BM,Es}}_{\text{EE}}\) would occur if an isotopic steady state were not reached for estrogen in the blood during the infusion of androgen. In such a situation,

\[
[p]^{\text{BM,Es}}_{\text{EE}} > [p]^{\text{BM,Es}}_{\text{EE}}
\]

Since urine is collected until essentially all radioactivity as estrogen is excreted, \([p]^{\text{BM,Es}}_{\text{EE}}\) will be independent of the isotopic steady state in blood for estrogen.

With this type of analysis, blood samples must still be analyzed to provide the data necessary for calculating blood production rates of androgens. These production rates will be needed to determine the mass of androgen converted to estrogen using the \([p]^{\text{BM,Es}}_{\text{EE}}\) values.

Results

Overall Aromatization. As shown in Table 1, the mean values for aromatization in men are greater than are the respective mean values in women, and in either sex the mean value for \([p]^{\text{BM,Es}}_{\text{EE}}\) is greater than that for \([p]^{\text{BM,Es}}_{\text{EE}}\). It should be noted that these studies were all done in subjects who were within 20% of their ideal weight.

These values are not different from the \([p]^{\text{BM,Es}}_{\text{EE}}\) and \([p]^{\text{BM,Es}}_{\text{EE}}\) values reported by MacDonald et al. (18). The similarity of the values in these normal subjects indicates that the previously mentioned step (Chart 1, Step 3) is not of major importance. In addition, Steps 1 and 2 appear to be relatively rapid, and an isotopic steady state was obtained during the infusion for the radiolabeled estrogen in the blood.

The sex difference in \([p]^{\text{BM,Es}}_{\text{EE}}\) values can be removed if the \([p]^{\text{BM,Es}}_{\text{EE}}\) values are normalized for body weight (Table 1). The mean values of \([p]^{\text{BM,Es}}_{\text{EE}}\), however, remain significantly different for men and women even if normalized for body weight. Therefore, we conclude that the sex hormone-binding globulin of testosterone may, in part, play a role in the difference in \([p]^{\text{BM,Es}}_{\text{EE}}\) values between men and women which cannot be explained by differences in body weight and/or composition.

The actual mass of androgen aromatized to a specific estrogen (\(P_{\text{BM}}^{\text{An,Es}}\)) can be calculated as

\[
[p]^{\text{BM,Es}}_{\text{EE}} = [p]^{\text{BM,Es}}_{\text{EE}} \times P_{\text{BM}}^{\text{An}}
\]

or

\[
[p]^{\text{BM,Es}}_{\text{EE}} = [p]^{\text{BM,Es}}_{\text{EE}} \times P_{\text{BM}}^{\text{An}}
\]

Table 1

<table>
<thead>
<tr>
<th></th>
<th>(n)</th>
<th>([p]^{\text{BM,Es}}_{\text{EE}})</th>
<th>([p]^{\text{BM,Es}}_{\text{EE}}) / kg</th>
<th>(n)</th>
<th>([p]^{\text{BM,Es}}_{\text{EE}})</th>
<th>([p]^{\text{BM,Es}}_{\text{EE}}) / kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men</td>
<td>15</td>
<td>0.0034 ± 0.0005</td>
<td>0.000047 ± 0.000007</td>
<td>14</td>
<td>0.0177 ± 0.0026</td>
<td>0.000024 ± 0.000004</td>
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<tr>
<td>Women</td>
<td>29</td>
<td>0.0018 ± 0.0001</td>
<td>0.000030 ± 0.000003</td>
<td>34</td>
<td>0.0107 ± 0.0008</td>
<td>0.000018 ± 0.000002</td>
</tr>
</tbody>
</table>

\(p^{b}\) <0.01 <0.02 <0.01 >0.1

\(p^{b}\) = Mean ± S.E.

\(p^{b}\) = \(p\) values for difference between men and women.
where $P_a^{E}$ is the blood production rate of the androgen. The relative importance of $P_a^{E_{ex}}$ to the production rate of the estrogen $P_a^E$ can be calculated as

$$\frac{P_a^{E_{ex}}}{P_a^E}$$

Although the fractional aromatization rates themselves are small, because of the magnitude of the androgen production compared to estrogen production rates, the amount of androgen aromatized will contribute substantially to the amount of estrogen produced. In men, this is the source of 60 to 75% of the estrogen produced each day. In normal young women, peripheral aromatization of androgens is not a source of estradiol and is only slightly more important as a source of estrone. In other situations, however, peripheral aromatization becomes far more important.

Aromatization in Specific Tissues. Much of the work on tissue aromatization has been carried out in vitro, in part due to the difficulty in sampling the venous drainage of multiple tissue in vivo, especially in humans. However, by catheterization of specific forearm veins draining muscle or draining adipose tissue, we have carried out studies on the aromatization of these tissues in normal men (16). We found aromatization to occur in both muscle and adipose tissue in the forearm at low but essentially equal fractional rates. We estimated the contribution of adipose and muscle tissue to the overall $[P]^{BM_{E1}}$ value using the overall blood flow to these tissues reported in the literature (11, 23). In this manner, we calculated that over 50% of the aromatization occurred in adipose tissue and muscle. It should be realized that these are rough estimates since they depend on homogeneity of tissue and calculated blood flows.

There are few data quantitating aromatization across other tissues in vivo. Preliminary studies in rhesus monkeys indicate that kidney, skin, and brain all contribute to the overall aromatization, but the contribution of the liver appears to be minimal.²

Conditions Associated with Altered Aromatization Rates. The first situation in which aromatization rates were noted to be increased was in the postmenopausal woman. Studies by Grodin et al. (8) and our own studies (13) (Table 2) indicated a marked increase in the aromatization of both androstenedione and testosterone in postmenopausal women as compared to premenopausal women. This increase appears to occur around the time of the menopause but does not appear to be age dependent. If one takes women of all ages, there is a correlation between the $[P]^{BM_{E1}}$ value and age. However, if one takes the premenopausal and postmenopausal groups separately, then for neither group is there a significant correlation between the $[P]^{BM_{E1}}$ value and age. The reason for the increase in peripheral aromatization in postmenopausal women remains uncertain. However, in this group, the major if not the only source for circulating estrogens is the peripheral aromatization of androgens (8, 13, 14).

Obesity is associated with a marked increase in aromatization (20), and morbidly obese individuals were noted to have $[P]^{BM_{E1}}$ values which ranged up to 10 times that in individuals of normal weight (5, 17). Of theoretical and practical interest is the fact that, in obese subjects, $[P]^{BM_{E1}}$ is considerably greater than $[P]^{BM_{E1}}$ where the latter is measured using the standard short infusion time (5). As the time of infusion is increased, then $[P]^{BM_{E1}}$ gradually rises to roughly equal $[P]^{BM_{E1}}$ (4). This is not due to an increase in Step 3 (Chart 1) but rather is due to the slow entry into the blood, via Step 2, of radiolabeled estrogen formed via Step 1 (4). As shown in Table 3, in individuals who are of normal weight,

$$[P]^{BM_{E1}} = [P]^{BM_{E1}}$$

but, in subjects who are overweight,

$$[P]^{BM_{E1}} < [P]^{BM_{E1}}$$

Therefore, in measuring $[P]^{BM_{E1}}$ or $[P]^{BM_{E1}}$ in obese subjects, the urinary method should be used.

Since adipose tissue is responsible for a considerable portion of the overall $[P]^{BM_{E1}}$ measured in blood or urine, it is not surprising that, as the adipose tissue mass increases, there is an increase in $[P]^{BM_{E1}}$. This increase is directly correlated to the increase in weight (5, 17, 20). Peripheral aromatization in obese men, as in normal men, is the major source of circulating estrogen and also becomes the major source of estrogens in many obese women (9, 17). From studies in vitro, Ackerman et al. (1) have shown that the stromal cell and not the adipocyte is the specific cell type containing the aromatase system.

In obese individuals who are studied before and immediately after losing weight, the $[P]^{BM_{E1}}$ remains the same (21). Whether this is related to a persistence of the stromal cells, an increase in blood flow (11), or other reasons remains to be determined.

Cirrhosis of the Liver. Men with cirrhosis of the liver frequently show signs of hyperestrogenism (3). Initially, this was thought to be due to a decrease in estrogen metabolism; however, recent studies have shown that cirrhotic males have

### Table 2

<table>
<thead>
<tr>
<th>$[P]^{BM_{E1}}$ and $[P]^{BM_{E2}}$ in young reproductive-age and older postmenopausal women</th>
<th>n</th>
<th>Age</th>
<th>$[P]^{BM_{E1}}$</th>
<th>$[P]^{BM_{E2}}$</th>
<th>p²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young women</td>
<td>29</td>
<td>26.9 ± 1.0</td>
<td>0.0016 ± 0.0001</td>
<td>&lt;0.01</td>
<td></td>
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<tr>
<td>Postmenopausal women</td>
<td>32</td>
<td>61.3 ± 1.4</td>
<td>0.0065 ± 0.0016</td>
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<td></td>
</tr>
<tr>
<td>Young men</td>
<td>34</td>
<td>29.2 ± 1.2</td>
<td>0.011 ± 0.0001</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>Postmenopausal men</td>
<td>45</td>
<td>65.6 ± 1.5</td>
<td>0.022 ± 0.002</td>
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<td></td>
</tr>
</tbody>
</table>

² p value for difference between young and postmenopausal women.

### Table 3

<table>
<thead>
<tr>
<th>Ratio of $[P]^{BM_{E1}}$ to $[P]^{BM_{E2}}$ in subjects of differing weights</th>
<th>Mean wt (% of ideal)</th>
<th>BM/BB⁹</th>
<th>$[P]^{BM_{E1}}/ [P]^{BM_{E2}}$</th>
<th>p³</th>
</tr>
</thead>
<tbody>
<tr>
<td>110 ± 3²</td>
<td>1.05 ± 0.13</td>
<td>4.34 ± 0.88</td>
<td></td>
<td></td>
</tr>
<tr>
<td>223 ± 23</td>
<td>0.0067 ± 0.0021</td>
<td></td>
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</tbody>
</table>

³ p value for difference between cirrhotics and normals.

### Table 4

<table>
<thead>
<tr>
<th>Peripheral aromatization in normal and cirrhotic men</th>
<th>$[P]^{BM_{E1}}$</th>
<th>$[P]^{BM_{E2}}$</th>
<th>$[P]^{BM_{E1}}$</th>
<th>$[P]^{BM_{E2}}$</th>
<th>p³</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cirrhotics</td>
<td>0.0482 ± 0.013²</td>
<td>0.0067 ± 0.0021</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normals</td>
<td>0.0177 ± 0.0026</td>
<td>0.0034 ± 0.0005</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

³ p value for difference between cirrhotics and normals.

² C. Longcope, R. Billiar, and B. Little, unpublished observations.

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Methods and Results of Aromatization Studies in Vivo

AUGUST 1982

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an increase in the peripheral aromatization of androgens (7). As our data indicate (Table 4), this increase is more marked for the aromatization of androstenedione than for testosterone. It is probable that the increase in sex hormone-binding globulin in cirrhotics (7) may inhibit the entry of testosterone into the tissues and thus render it less available as a substrate for aromatization, but androstenedione, which is not bound to the sex hormone-binding globulin, would all be available to the tissues. The failure to find a high degree of aromatization in normal hepatic tissues raises an interesting point as to whether the increase in aromatization in hepatic cirrhosis in individuals is due to an increase in aromatization in the liver itself or to an increase in aromatization in peripheral, nonglandular tissue. Since blood flow is increased in peripheral tissues in many instances of cirrhosis (24), it is probable that this increased blood flow results in more androgen substrate available to the tissues and therefore greater aromatization and that the liver contributes little to the increase in aromatization.

**Cancer.** The fractional rate of aromatization has been examined in various endocrine-related cancers such as endometrial carcinoma and breast carcinoma. Although initial reports suggested an increased aromatization in women with endometrial cancer compared to normal women, MacDonald et al. (17) and Rizkallah et al. (20) have shown that, in women with endometrial cancer, when matched for weight and menopausal status, the aromatization rates are similar. Studies in breast cancer have given equivalent results (19, 21), and there appears to be no direct increase in aromatization related to breast cancer. Although breast cancer tissue appears to contain the aromatase (12) system, the contribution of the cancer to the overall aromatization rate would appear too low to alter that rate.

**Miscellaneous Conditions.** Initial studies by Southren et al. (22) in subjects with hyperthyroidism suggested that there was an increase in peripheral aromatization in this disorder. However, in recent studies, we have not been able to confirm this finding by measurements in either blood or urine. Further work may be necessary to clarify this point. Rizkallah et al. (20) reported an increase in [\(\triangle 7\)] progesterone in a small group of postoperative patients. The reason for this is uncertain.

**Control Mechanisms.** Since aromatization by peripheral tissues is increased in a number of disparate conditions, we were interested in factors which might control the peripheral aromatization. Accordingly, we tested, in rhesus monkeys, a number of hormones and, as noted in Table 5, we were unable to influence the aromatization to any significant degree (6). It should be noted that estrogen administration to male monkeys did result in a decline in the fractional aromatization, but this decline was not significant due to the wide range of individual values and the small number of animals involved. However, there appears to be no indication that gonadotropins or other polypeptide hormones influence peripheral aromatization directly. This lack of influence is interesting in view of the marked increases in gonadotropins and the fractional aromatization rates which occur at the menopause.

Thus, aromatization appears to occur in many tissues, but primarily in adipose tissue and muscle, and can be a major source of circulating estrogens. Increases in the aromatization rate can be present in various diseases and can result in signs of hyperestrogenism in these individuals. Mechanisms underlying control of the peripheral aromatization are still obscure, but blood flow and availability of androgen substrate to specific tissues may play a critical role in this control.

### References


### Table 5

<table>
<thead>
<tr>
<th>Treatment</th>
<th>([1]^{1})</th>
<th>([2]^{2})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.015 ± 0.001</td>
<td>0.0024 ± 0.003</td>
</tr>
<tr>
<td>Human chorionic gonadotropin</td>
<td>1000 units each day for 5 days</td>
<td>0.0095 ± 0.0035</td>
</tr>
<tr>
<td>Follicle-stimulating hormone</td>
<td>500 units each day for 5 days</td>
<td>0.016 ± 0.004</td>
</tr>
<tr>
<td>Human chorionic gonadotropin + follicle-stimulating hormone</td>
<td>1000 + 500 units</td>
<td>0.013 ± 0.0032</td>
</tr>
<tr>
<td>Estrogen</td>
<td>100 µg each day for 8 days</td>
<td>0.015 ± 0.006</td>
</tr>
<tr>
<td>Castrate</td>
<td>3 wk</td>
<td>0.008 ± 0.001</td>
</tr>
<tr>
<td>Testosterone</td>
<td>5 mg each day for 7 days</td>
<td>0.0021 ± 0.0005</td>
</tr>
<tr>
<td>Estrogen</td>
<td>100 µg twice a wk for 2 wk</td>
<td>0.0009 ± 0.0005</td>
</tr>
</tbody>
</table>

\(^{a}\) Mean ± S.E.
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