Continuous infusions of Δ4-androstenedione-7,3H and testosterone-7,3H have been used to demonstrate that these androgens are converted to estrone and 17β-estradiol, and contribute to the circulating blood levels of these estrogens in normal males and females. The conversion ratio (ratio of concentrations of radioactivity of free product steroid [χ^PRO] and free precursor steroid [χ^PRE], both corrected for recoveries, after an infusion of radioactive precursor steroid) for androstenedione (precursor) to estrone (product) is 0.013 in males and 0.007 in females, and the conversion ratio for testosterone (precursor) to estradiol (product) is 0.0018 in males and 0.005 in females. The transfer constant, [ρ]_{BB}^{AE1}, for androstenedione conversion to estrone ([ρ]_{BB}^{AE1} = per cent of infused androstenedione, precursor, converted to estrone, product, when infusion and measurement are both in blood) is 1.35% in males and 0.74% in females, and the transfer constant, [ρ]_{BB}^{TE2}, for testosterone conversion to estradiol is 0.39% in males and 0.15% in females.

Whether measured as conversion ratio or transfer constant, the peripheral aromatization of androstenedione takes place to a greater degree than that of testosterone, and, for the respective androgens, both the conversion ratio and [ρ]_{BB} value are greater in males than females.

For the androgen interconversions, [ρ]_{BB}^{AT} is 4.5% in males and 2.2% in females; [ρ]_{BB}^{TA} is 8.2% in males and 12.0% in females.

Studies on the distribution coefficients (effective […]

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**Conversion of Blood Androgens to Estrogens in Normal Adult Men and Women**

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**ABSTRACT** Continuous infusions of Δ1-androstenedione-7,3H and testosterone-7,3H have been used to demonstrate that these androgens are converted to estrone and 17β-estradiol, and contribute to the circulating blood levels of these estrogens in normal males and females. The conversion ratio (ratio of concentrations of radioactivity of free product steroid [P] and free precursor steroid [P*], both corrected for recoveries, after an infusion of radioactive precursor steroid) for androstenedione (precursor) to estrone (product) is 0.013 in males and 0.007 in females, and the conversion ratio for testosterone (precursor) to estradiol (product) is 0.0018 in males and 0.005 in females. The transfer constant, $[P]_{A}^{m}$, for androstenedione conversion to estrone ($[P]_{A}^{m}$ = per cent of infused androstenedione, precursor, converted to estrone, product, when infusion and measurement are both in blood) is 1.35% in males and 0.74% in females, and the transfer constant, $[P]_{A}^{en}$, for testosterone conversion to estradiol is 0.39% in males and 0.15% in females.

Whether measured as conversion ratio or transfer constant, the peripheral aromatization of androstenedione takes place to a greater degree than that of testosterone, and, for the respective androgens, both the conversion ratio and $[P]_{m}$ value are greater in males than females.

For the androgen interconversions, $[P]_{m}$ is 4.5% in males and 2.2% in females; $[P]_{en}$ is 8.2% in males and 12.0% in females.

Studies on the distribution coefficients (effective concentration in red cells/plasma) for precursor radioactivity were also made. In both males and females the distribution coefficient for androstenedione is 0.16–0.17 while that of testosterone is 0.01–0.03.

**INTRODUCTION**

In vivo studies in humans using isotopically labeled Δ1-androstenedione and testosterone have shown that interconversions occur between these steroids, both of which are secreted (1, 2). The contribution of the chemical product of these interconversions to the blood pool of free steroid is less than the contribution to the pool of steroid metabolites appearing in the urine. Thus, measurement of production and secretion rates from analysis of urinary metabolites can lead to erroneous conclusions about blood production and secretion rates (2–4).

Similarly, the estrogens, estrone and 17β-estradiol, are interconvertible (5–7) and these conversions are different when calculated from measurements made from urinary metabolites as opposed to measurements made from the free steroids in blood (6–8).

It has been demonstrated that androstenedione and testosterone can be aromatized and that they contribute to the urinary metabolites of estrone and estradiol (9–11). However, if the conversions of androstenedione and testosterone to estrone and estradiol occur primarily in tissues such as the liver, where further metabolism and/or conjugation takes place, these conversions need not necessarily contribute significantly to the blood production of estrogens. Therefore, to measure the conversions which contribute significantly to the blood production of estrogens, it is necessary to measure the free steroid radioactivity in the peripheral blood after administering the androgen into that compartment.

This paper presents a quantitative analysis of androgen-estrogen conversion as measured in blood of normal men and women using the continuous infusion technique (12).
METHODS

Normal adult volunteers were infused with testosterone-7α-\(^{3}H\) or \(\Delta^{4}\)-androstenedione-7α-\(^{3}H\). The adults were 21-40 yr of age, had given informed consent, and were in excellent health taking no medication. All the females infused were in the follicular phase of the menstrual cycle.

The solvents used for extraction of plasma samples and for chromatography were of spectral quality. Pyridine, acetic anhydride, and pipsyl chloride were obtained and purified as described (7, 13).

Testosterone-7α-\(^{3}H\) (SA 10.0 c/mmole) and \(\Delta^{4}\)-androstenedione-7α-\(^{3}H\) (1.0 c/mmole), obtained from Nuclear Chicago Corporation, Des Plaines, Ill. and testosterone-4-\(^{14}C\) (50 mc/mmole) and \(\Delta^{4}\)-androstenedione-4-\(^{14}C\) (50 mc/mmole) obtained from New England Nuclear Corp., Boston, Mass. were purified by column chromatography (2). 17β-Estradiol-4-\(^{14}C\) (31.7 mc/mmole) and estrone-4-\(^{14}C\) (33.7 mc/mmole) were obtained from Nuclear Chicago Corp. and purified by column and paper chromatography as described (7).

The paper chromatography of samples was carried out using Whatman 2 paper in the following systems:

- P-1 stationary phase, methanol : water, 85:15 mobile phase, Skellysolve C
- P-2 stationary phase, methanol : water, 90:10 mobile phase, hexane
- P-3 stationary phase, methanol water, 90:10 mobile phase, isooctane
- P-4 stationary phase, water 90:10 mobile phase, ligroin

Thin-layer chromatography (TLC) was carried out using Neutral Alumina GF254 (E. Merck, A. G., Darmstadt, Germany), Silica Gel HF254 or GF254 (Brinkmann Instruments, Inc., Great Neck, N. Y.) in the following systems:

- C-1 benzene : ethyl acetate, 2:1
- C-2 chloroform : acetone, 89:11
- C-3 chloroform acetone, 92:8
- C-4 chloroform : acetone, 98:2
- C-5 cyclohexane : ethyl acetate, 75:25
- C-6 chloroform : methanol : 1 N-NaOH, 9:2:11
- C-7 benzene : methanol, 98:2
- C-8 benzene : ethanol, 98:2
- C-9 cyclohexane : ethyl acetate, 75:50

All experiments were started between 8 and 9 a.m., with the subjects fasting and in the supine position 1 hr before the loading dose was administered. At zero time an injection of either 4-6 mc of androstenedione-7α-\(^{3}H\) or testosterone-7α-\(^{3}H\) in 10 ml isotonic saline was given into an arm vein. 30 min later, an infusion of the same steroid was begun and continued at a constant rate for 120 min. The infusion consisted of 10-30 mc of the \(^{3}H\)-labeled steroid in 50 ml of an 8% ethanolic solution of normal saline administered through Teflon tubing (Becton-Dickinson & Co., Rutherford, N. J.) so that absorption was negligible. Samples were taken from both the stock solution and from the infusion tubing for calculation of radioactivity infused (14).

Blood samples of 50-60 cc were obtained at 120, 135, and 150 min after the loading dose and were drawn into heparinized syringes from the opposite arm vein. Blood was centrifuged and the plasma extracted immediately or frozen at −20°C until extracted.

Analysis of plasma. The plasma was added to a flask containing androstenedione-4-\(^{14}C\) (350 dpm), testosterone-4-\(^{14}C\) (350 dpm), estrone-4-\(^{14}C\) (140 dpm), and estradiol-4-\(^{14}C\) (140 dpm) and 50 µg each of the following nonradioactive steroids: androstenedione, testosterone, estrone, and estradiol. To this was added 0.5 ml 4 N NaOH; the contents were then swirled and extracted with three volumes and then two volumes of anhydrous ether (2). The ether extracts were pooled and washed twice with 10-ml volumes of distilled water. The ether was removed under vacuum and the residue transferred to a stoppered tube with three 10 ml volumes of 70% ethanol. 12 ml of petroleum ether (bp 75.8-96.3°C) was added, the tube was shaken, centrifuged, and the petroleum ether removed and discarded. The aqueous methanol was removed under vacuum and the residue taken up in 4 ml benzene and partitioned against 20 ml 1 N NaOH. The tube was centrifuged and the benzene fraction containing the androgens removed. To the 1 N NaOH was added another 1 ml benzene, the tube was shaken, centrifuged, and the benzene removed and pooled. The benzene fraction containing the androgens and the NaOH fraction containing the estrogens were separately treated as described.

Benzone fraction (androgens). The benzene was removed, the residue taken up in 5 ml dichloromethane (CH2Cl2) and washed with 1.5 ml 0.1 M acetic acid and then with two 1 ml volumes of H2O. The CH2Cl2 was removed under vacuum and the residue run on alumina TLC in system C-1. The androstenedione and testosterone spots, located using 254 m\(\mu\) UV light, were separately eluted as described (2). The dried samples containing androstenedione and testosterone from TLC C-1 were spotted on paper and run in system P-1. The steroids again were located with UV light at 254 m\(\mu\), and the appropriate areas were eluted into counting vials, and the vials dried, and counted as described (15). Recoveries through the procedure were 30-45% for androstenedione and 45-65% for testosterone.

NaOH extracts (estrogens). The NaOH extracts were brought to pH 7 with glacial acetic acid and extracted twice with 50 ml of anhydrous ether. The ether extracts were washed twice with 10 ml of H2O, and dried, and run in parallel with nonradioactive estrone and 17β-estradiol on Silica Gel HF254 in system C-2. The carrier estrogens were located and eluted as described (7). After elution, the tube containing estradiol was dried and the residue rerun on Silica Gel HF254 in system C-6. The carrier estrone was located and eluted, and the tubes containing estradiol from C-6 and estrone from C-2 were dried and the contents acetylated overnight (7). Under these conditions, estrone was converted into estrone-3-acetate and estradiol was converted into estradiol-3-monoacetate. Estrone acetate was then chromatographed on Silica Gel HF254 in two dimensions in system C-4. Estradiol-3-monoacetate was chromatographed on Silica Gel HF254 in two dimension using systems C-3 and then C-7. The steroids were located and eluted into counting vials. These were dried and 10 ml of scintillation fluid (84 ml Liquidifluor [Pilot Chemicals, Inc., Watertown, Mass.], 20 ml of ethanol, and 1 liter of toluene) were added to each vial. The vials were counted for 10 X 40 min. Using a treatment similar to that described by Horton and Tait (15), it was possible to calculate maximum counting errors. For the determination of the concentration of radioactivity in the estrone from androstenedione in both sexes and in the estradiol from testosterone in males the maximal counting errors were 10%, and in the estradiol from testosterone in females the maximal counting errors were 25%.

The recoveries from plasma through the entire purification were 35-50% for estrone-4-\(^{14}C\) and 40-60% for estradiol-4-\(^{14}C\). Analysis of whole blood. In certain experiments, a 10 cc aliquot of whole blood was removed before centrifugation. Androstenedione-4-\(^{14}C\) (350 dpm) and testosterone-4-\(^{14}C\)
Radiochemical purity of recovered steroids. The radiochemical purity of the recovered androstenedione and testosterone has already been demonstrated (2).

The following methods were used to establish the radiochemical purity of the recovered estrone. (a) After an infusion of androstenedione, using three times the usual amount of androstenedione-\textsuperscript{2}H, blood was drawn at the usual time intervals, and the plasma with \textsuperscript{14}C indicator and carrier steroids were pooled and extracted as usual. The estrone fraction was carried through the usual chromatography steps, and, after the two-dimensional TLC of estrone acetate, one-third was taken for counting. The remaining two-thirds was reduced with sodium borohydride (7) and the resultant estradiol 3-monoacetate was chromatographed on paper in system P-2. Nonradioactive steroid, run in parallel with the samples, was sprayed with 10% phosphomolybdic acid in MeOH to locate the steroid. The area corresponding to authentic estradiol 3-monoacetate was recovered, eluted into a counting vial, and counted as described. The \textsuperscript{3}H: \textsuperscript{14}C ratio (±statistical counting error) of the estrone acetate was 2.46 ±0.03 and of the estradiol 3-monoacetate was 2.57 ±0.03.

(b) After a similar infusion of androstenedione, plasma samples were pooled and extracted as usual. The estrone fraction was carried through the usual chromatography steps and after the two-dimensional TLC of estrone acetate, one-third was taken for counting. The remainder was reduced with sodium borohydride and the resultant estradiol 3-monoacetate was spotted on Silica Gel GF\textsubscript{254} and run in two dimensions using system C-3 and C-7. The area corresponding to authentic estradiol 3-monoacetate was eluted and acetylated overnight with pyridine, 0.1 ml, and 10% acetic anhydride in benzene, 0.1 ml. The reaction was stopped with 0.05 ml ethanol, and the resulting estradiol diacetate was chromatographed on Silica Gel in system C-5. The area corresponding to authentic estradiol diacetate was eluted into a counting vial and counted. The \textsuperscript{3}H: \textsuperscript{14}C ratio of the estrone acetate was 0.96 ±0.19 and of the estradiol diacetate was 1.09 ±0.20. The similarity of the \textsuperscript{3}H: \textsuperscript{14}C ratio in these two experiments indicates that a high degree of radiochemical purity results from the routine purification procedure.

For the demonstration of radiochemical purity of the recovered estradiol, the following was done. (a) After an infusion of testosterone-\textsuperscript{3}H (T-\textsuperscript{3}H), using twice the usual amount of T-\textsuperscript{3}H, blood was drawn at 120, 135, and 150 min, and the plasma volumes with indicator and carrier were pooled and extracted. The estradiol fraction was carried through the usual chromatographic steps and after the two-dimensional TLC of estradiol 3-monoacetate, one-third was taken for counting. The remaining two-thirds was acetylated overnight with 0.1 ml pyridine and 0.1 ml 10% acetic anhydride in benzene. The reaction was stopped with 0.1 ml ethanol. The tube was dried and the residue spotted on Silica Gel GF\textsubscript{254} and run in system C-5. The estradiol diacetate spot was located under 254 mA light, eluted into a counting vial, and counted. The \textsuperscript{3}H: \textsuperscript{14}C ratios of the estradiol 3-monoacetate and estradiol diacetate were 0.22 ±0.02 and 0.23 ±0.01, respectively.

(b) After another infusion of testosterone-\textsuperscript{3}H, three times the usual amount, the pooled plasma recovered from the three blood samples was extracted. The estradiol fraction was analyzed and after the two-dimensional TLC, one-third of the resultant estradiol 3-monoacetate was counted. The remaining two-thirds were chromatographed in paper system P-2. Standards were run along with the sample and sprayed with 10% phosphomolybdic acid in methanol to locate the steroids. The area corresponding to authentic estradiol 3-monoacetate was recovered and eluted into a tube. The tube was dried and the residue was acetylated overnight in the dark with 0.1 ml pyridine and 0.1 ml 10% acetic anhydride in benzene. The reaction was stopped with 0.1 ml ethanol, the tube was dried, and the residue was spotted on Silica Gel H\textsubscript{F-254} and run in system C-5. The estradiol diacetate was located as usual, eluted, and counted. The \textsuperscript{3}H: \textsuperscript{14}C ratios of the estradiol 3-monoacetate and estradiol diacetate were 0.43 ±0.02 and 0.45 ±0.01, respectively.

(c) After a 3rd infusion of testosterone, using three times the usual amount, the pooled plasma from the three blood samples was extracted. After the 2nd TLC of the estradiol fraction, a portion was taken, acetylated to form estradiol 3-monoacetate, chromatographed as usual in two dimensions, and counted. The remainder of the estradiol was dried and dissolved in a solution of 25 μl aceton, 25 μl H\textsubscript{2}O, and 25 μl 0.1 N NaOH (13). While shaking, 0.2 mg pipsyl chloride was added to this. The sides of the tube were washed with 25 μl aceton, and the tube was shaken for 30 min at room temperature. Then, 0.4 ml chloroform was added and washed three times with 0.25 ml volumes of H\textsubscript{2}O. The tube was dried under N\textsubscript{2}, the residue spotted with carrier on Silica Gel GF\textsubscript{254} and run in two dimensions in systems C-9 and C-8. The estradiol 3-monopipsylate was located with 254 μA UV light and eluted. The monopipsylate was then spotted on paper and run in system P-4. It was then eluted and respotted on paper. To the spot was added 2 drops of 5% chromic acid in water to form estrone-monopipsylate. The paper was dried in air for 10 min and then run in system P-3. The estrone-monopipsylate was reduced using sodium borohydride, and the resultant estradiol 3-monopipsylate was acetylated overnight with 0.3 ml pyridine and 0.15 ml acetic anhydride. The tube was dried under vacuum, the residue spotted on Silica Gel GF\textsubscript{254} and run in system C-5. The steroid was located with 254 μA UV light, eluted, and counted. The \textsuperscript{3}H: \textsuperscript{14}C ratios of the estradiol 3-monoacetate and estradiol 3-monopipsylate-17-monoacetate were 0.38 ±0.01 and 0.42 ±0.05.

The stability of the \textsuperscript{3}H: \textsuperscript{14}C ratio through various derivatization and chromatography steps indicates the radiochemical purity of the estradiol-\textsuperscript{3}H recovered after testosterone-\textsuperscript{3}H infusion. Proof that a steady state has been reached for radioactivity as the precursor and product steroids. It has been shown (2) that under similar conditions of infusion, the concentrations of radioactivity as testosterone and \Delta\textsuperscript{4}-androstenedione, whether as precursor of product, have reached a steady state. When estrone is the product, and the concentration of radioactivity as estrone in the 135 min sample is set at 100%, the concentration of radioactivity at 120 min and 150 min are 92 ±5% (statistical counting error) and 101 ±3% of this value. Neither of these differ significantly from 100% (P > 0.05).

Similarly, when the concentration of radioactivity as estradiol at 135 min is set at 100%, the concentrations at 120 min and 150 min are 87 ±10% and 103 ±22% of this value. Neither of these differ significantly from 100% (P > 0.05). It has been reported (7) that when estrone-\textsuperscript{3}H or estradiol-\textsuperscript{3}H are administered under similar conditions of infusion the concentrations of radioactivity in both precursor and product estrogen reach a steady state within 2 hr. In addition when estrone-\textsuperscript{3}H or estradiol-\textsuperscript{3}H are administered under conditions similar to that described in the present...
paper but for infusion periods ranging from 6 to 12 hr, the concentrations of radioactivity in the blood do not vary significantly after 2 hr.\(^1\)

This indicates, therefore, that a steady state for both precursor androgen and product estrogen radioactivity has been reached under the conditions used in the present study. The variations noted between the 120 min and 150 min values for product estrogens largely reflect counting errors and not the failure to reach a steady state.

**Definitions and statistical approach.** The conversion ratio (\(\Delta R_{\text{BB}}^{\text{PRE-PRO}}\)) is the ratio of the concentrations of the radioactivity in the free product steroid (\(\Delta F_{\text{PRO}}\)) and free precursor steroid (\(\Delta F_{\text{PRE}}\)), both corrected for recoveries, after an infusion of radioactive precursor steroid (2, 7).

As such, it is a direct measurement obtained in each individual subject.

For \(\Delta R_{\text{BB}}^{\text{PRE-PRO}}\) in both males and females, and for \(\Delta R_{\text{BB}}^{\text{PRE-PRO}}\) in males, the counting errors for measurement of product radioactivity were 10%, and the counting errors for measurement of precursor radioactivity were 2%. Errors in pipetting would contribute a further 3% so that the propagated error (16) for these determinations would be \(\pm 11\%\). For \(\Delta R_{\text{BB}}^{\text{PRE-PRO}}\) in females, the counting errors for the product were 25%, and for the precursor 2%. Including pipetting errors, the total error for this determination would be \(\pm 26\%\).

The transfer constant, \([p]_{\text{BB}}^{\text{PRE-PRO}}\) of precursor conversion to product, originally described by Gurpide, MacDonald, Vande Wiele, and Lieberman (17) and adapted by Horton and Tait (2) for measurements in blood, is defined as the per cent of precursor converted to product when precursor infusion and product measurement are in peripheral blood. As such, it is calculated as \((\Delta C_{\text{PRO}}^{\text{X}} \times \Delta F_{\text{PRE}}^{\text{X}})/(\Delta C_{\text{PRE}}^{\text{X}})\times 100\) where \(\Delta C_{\text{X}}\) is the rate of infusion of radioactivity as precursor. Since by definition (18) \(\Delta C_{\text{BB}}^{\text{PRE-PRO}} = \Delta F_{\text{BB}}^{\text{PRE-PRO}}\),

\[
[p]_{\text{BB}}^{\text{PRE-PRO}} = \frac{\Delta C_{\text{PRO}}}{\Delta C_{\text{PRE}}} \times \Delta F_{\text{PRE}}^{\text{X}} \times 100.
\]

The term

\[
\Delta C_{\text{PRO}}/\Delta C_{\text{PRE}} \times \Delta F_{\text{PRE}}^{\text{X}}
\]

is obtained from one infusion of radioactive precursor. \(\Delta C_{\text{PRO}}\) may be obtained either by a separate infusion or by the simultaneous infusion of both precursor and product which are labeled with different isotopes. In the past (2, 7, 15), \([p]_{\text{BB}}^{\text{PRE-PRO}}\) has been calculated using a mean value for \(\Delta C_{\text{PRO}}\) obtained from a group of individuals who are similar as to sex, age, and health to the group infused with precursor, and a similar approach was used in the present study. For the calculations of \([p]_{\text{BB}}^{\text{AT}}\) and \([p]_{\text{BB}}^{\text{TA}}\) the \(\Delta C_{\text{BB}}^{\text{AT}}\) and \(\Delta C_{\text{BB}}^{\text{TA}}\) values are those reported by Longcope, Layne, and Tait (7) in individuals similar as to age, sex, and health to the groups discussed in the present study. For the calculations of \([p]_{\text{BB}}^{\text{AT}}\) and \([p]_{\text{BB}}^{\text{TA}}\), the \(\Delta C_{\text{BB}}^{\text{AT}}\) and \(\Delta C_{\text{BB}}^{\text{TA}}\) values are those obtained in the present study. Since it has been shown that MCR's of the androgens and estrogens are correlated with body surface area (7, 19), we have used \(\Delta C_{\text{PRO}}\) and \(\Delta C_{\text{PRE}}\) corrected for body surface area to calculate \([p]_{\text{BB}}\) values. Thus, an individual \([p]_{\text{BB}}^{\text{PRE-PRO}}\) value is the product of a mean value, MCR PRO, multiplied by an individual value, \(\Delta C_{\text{PRO}}/(\Delta C_{\text{PRE}} \times \Delta F_{\text{PRE}}^{\text{X}})\). Even though it can be assumed the values for MCR PRO and \(\Delta C_{\text{PRO}}/(\Delta C_{\text{PRE}} \times \Delta F_{\text{PRE}}^{\text{X}})\) have a normal distribution, the product \([p]_{\text{BB}}^{\text{PRE-PRO}}\) of two such normal distributions may not necessarily have a normal distribution. However, functions of a normal distribution are normally distributed (20), and since the sum of two normal distributions has a normal distribution (21), we have used the natural logarithmic function for both MCR PRO and \(\Delta C_{\text{PRO}}/(\Delta C_{\text{PRE}} \times \Delta F_{\text{PRE}}^{\text{X}})\) to calculate \([p]_{\text{BB}}^{\text{PRE-PRO}}\). Statistical analyses have then been carried out on the logarithms. Mean values for \([p]_{\text{BB}}\) are expressed, however, as the antilog of the mean of the logarithmic functions. These \([p]_{\text{BB}}\) values will then be geometric means and are given along with their \(95\%\) confidence limits. The geometric mean differs, however, by less than 2% from the respective arithmetic mean.

Since an individual \([p]_{\text{BB}}\) value is calculated from a mean MCR PRO value and an individual \(\Delta C_{\text{PRO}}/(\Delta C_{\text{PRE}} \times \Delta F_{\text{PRE}}^{\text{X}})\) value, the propagated error is difficult to determine. The theoretical error in the mean logarithmic \([p]_{\text{BB}}^{\text{PRE-PRO}}\) value can be obtained from the standard deviations of the means for the natural logarithms of MCR PRO and \(\Delta C_{\text{PRO}}/(\Delta C_{\text{PRE}} \times \Delta F_{\text{PRE}}^{\text{X}})\). The maximal error is then 18% of the mean logarithm of \([p]_{\text{BB}}^{\text{PRE-PRO}}\).

**RESULTS**

Radioactivity as precursor and product. In Tables I and II are listed the concentrations (counts per minute per liter), corrected for recoveries, of androgen precursor and estrogen product. When androstenedione-\(^{15}\)H was infused as precursor, estrone-\(^{15}\)H was always recovered as product. In only three instances, subjects 6, 13, and 15, were we able to recover \(^{15}\)H-labeled estradiol. In these three subjects, the amounts of radioactivity infused had been two to three times greater (40–50 mc/2 hr) than the usual amounts infused. The mean values for estradiol were, respectively, 160, 100, and 120 cpm/liter.

When testosterone-\(^{15}\)H was infused as precursor, estradiol-\(^{15}\)H was always recovered as product. In only two instances, subjects 4 and 6, was estrone-\(^{15}\)H recovered as product. In these two subjects, the amounts of radioactivity infused had been roughly two times (30–40 mc/2 hr) more than the usual, and the mean values for estrone-\(^{15}\)H were 50 and 120 cpm/liter, respectively.

Conversion ratios (\(\Delta CR = \text{mean} \pm \text{SEM}\)) for androgen conversion to estrogen. In nine males, the mean \(\Delta CR_{\text{BB}}\) was 0.013 \(\pm 0.0024\) (SEM), and in six females, the mean \(\Delta CR_{\text{BB}}\) was 0.0069 \(\pm 0.0007\) (Table I). \(\Delta CR_{\text{BB}}\) was 0.002 in the one male in whom it was possible to measure estradiol-\(^{15}\)H. In the females, subjects 13 and 15, \(\Delta CR_{\text{BB}}\) was 0.0007 and 0.0014, respectively.

In eight males, the mean \(\Delta CR_{\text{BB}}\) was 0.0018 \(\pm 0.0002\), and, in six females, the mean \(\Delta CR_{\text{BB}}\) was 0.0005 \(\pm 0.0001\) (Table II). \(\Delta CR_{\text{BB}}\) was 0.001 in the two subjects, both males, in whom it could be measured directly.

\([p]_{\text{BB}}\) values for androgen conversion to estrogen. In Tables III and IV are listed the individual and mean \([p]_{\text{BB}}\) values with the 95% confidence limits given in parentheses, mean (95% confidence limits). In nine males, the mean \([p]_{\text{BB}}\) value was 1.33 (1.01–1.76) %

\(^1\)Longcope, C. Unpublished data.
**Table I**

Data for the Determination of (±PRO)/(±PRE) for Androstenedione Conversion to Estrone

<table>
<thead>
<tr>
<th>Subject</th>
<th>Sex</th>
<th>Infused androstenedione-H</th>
<th>Androstenedione (±PRE), min after priming dose</th>
<th>Estrone (±PRO), min after priming dose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>cpm/day</td>
<td>cpm/liter plasma‡</td>
</tr>
<tr>
<td>1</td>
<td>M</td>
<td>95.2 × 10^4</td>
<td>42,900</td>
<td>37,500</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>82.3 × 10^4</td>
<td>32,400</td>
<td>34,400</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>77.7 × 10^4</td>
<td>27,800</td>
<td>24,900</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>74.2 × 10^4</td>
<td>32,600</td>
<td>31,200</td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>86.1 × 10^4</td>
<td>42,700</td>
<td>51,700</td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>266.0 × 10^4</td>
<td>POOL</td>
<td>—</td>
</tr>
<tr>
<td>7</td>
<td>M</td>
<td>101.5 × 10^4</td>
<td>49,300</td>
<td>—</td>
</tr>
<tr>
<td>8</td>
<td>M</td>
<td>118.4 × 10^4</td>
<td>—</td>
<td>59,300</td>
</tr>
<tr>
<td>9</td>
<td>M</td>
<td>114.5 × 10^4</td>
<td>38,700</td>
<td>46,300</td>
</tr>
</tbody>
</table>

**Mean ± SE**

0.0127 ± 0.0024

* (±PRO)/(±PRE) = CRBBABR.

†H (counts per minute per liter) corrected for losses with ¹⁴C indicator.

**Table II**

Data for Determination of (±PRO)/(±PRE) for Testosterone Conversion to Estradiol

<table>
<thead>
<tr>
<th>Subject</th>
<th>Sex</th>
<th>Infused testosterone-H</th>
<th>Testosterone (±PRE), min after priming dose</th>
<th>Estradiol (±PRO), min after priming dose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>cpm/day</td>
<td>cpm/liter plasma‡</td>
</tr>
<tr>
<td>1</td>
<td>M</td>
<td>62.1 × 10^4</td>
<td>63,200</td>
<td>65,900</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>45.9 × 10^4</td>
<td>48,200</td>
<td>47,200</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>94.0 × 10^4</td>
<td>110,700</td>
<td>119,800</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>116.6 × 10^4</td>
<td>135,500</td>
<td>152,600</td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>70.2 × 10^4</td>
<td>110,100</td>
<td>126,600</td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>184.1 × 10^4</td>
<td>—</td>
<td>182,400</td>
</tr>
<tr>
<td>7</td>
<td>M</td>
<td>204.3 × 10^4</td>
<td>POOL</td>
<td>172,400</td>
</tr>
<tr>
<td>8</td>
<td>M</td>
<td>110.2 × 10^4</td>
<td>94,200</td>
<td>95,900</td>
</tr>
</tbody>
</table>

**Mean ± SE**

0.0018 ± 0.0002

* (±PRO)/(±PRE) = CRBBABR.

†H (counts per minute per liter) corrected for losses with ¹⁴C indicator.

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and in six females, the mean $[p]_{BB}^{AB}$ value was 0.74 (0.59–0.92)% (Table III). In the one male and two females in whom $[p]_{BB}^{AB}$ values were measurable, the values were 0.07, 0.07, and 0.08%, respectively.

In eight males, the mean $[p]_{BB}^{AB}$ value was 0.38 (0.32–0.45)% and in six females the mean $[p]_{BB}^{AB}$ value was 0.16 (0.10–0.21)% (Table IV). For the two males in whom $[p]_{BB}^{AB}$ was measurable, the value was 0.13% in both.

Metabolic clearance rates, CRAB, and $[p]_{BB}$ values for androstenedione and testosterone. As noted in Table III, for the nine males, the mean MCR$^A$ was 2210 ±130 liters/day and 1125 ±70 liters/day per m$^2$. Neither of these values is significantly different ($P > 0.10$) than the mean MCR$^A$ for the six females of 2020 ±140 liters/day and 1210 ±70 liters/day per m$^2$. The mean values for MCR$^A$ in eight males (Table IV) of 930 ±70 liters/day and 465 ±45 liters/day per m$^2$ are both significantly ($P < 0.01$) greater than the respective mean values for MCR$^A$ in six females of 440 ±50 liters/day and 275 ±30 liters/day per m$^2$.

In Table V are shown the CRAB and $[p]_{BB}$ values. In nine males, the mean CRAB$^A$ was 0.12 ±0.01 and $[p]_{BB}^{AB}$ was 4.5 (3.7–5.5)% in six females, CRAB$^A$ was 0.10 ±0.008 and $[p]_{BB}^{AB}$ was 2.3 (1.7–2.9)%.

In eight males, the mean CRAB$^A$ was 0.034 ±0.003 and the mean $[p]_{BB}^{AB}$ was 8.2 (6.2–11.0)% in six females, the mean CRAB$^A$ was 0.027 ±0.002 and the mean $[p]_{BB}^{AB}$ was 12.0 (9.6–15.0)%.

Distribution coefficient for precursor radioactivity between red cells and plasma (Table VI). In certain experiments whole blood as well as plasma was analyzed for the concentration of radioactivity as the free precursor. It was then possible to calculate the distribution coefficient (effective concentration in red cells/plasma) for androstenedione and testosterone according to the following formula (7):

$$\frac{x^B}{x^P} = \frac{1 - Hct}{Hct}.$$

Where $x^A$ and $x^B$ represent the concentration of radioactivity in whole blood and plasma and Hct represents the hematocrit as determined by the Wintrobe method (22). The results are given in Table VI, and the mean distribution coefficient does not differ between males.

**Table III**

<table>
<thead>
<tr>
<th>Subj.</th>
<th>Sex</th>
<th>MCR$^A$</th>
<th>MCR$^A$</th>
<th>Ln CRAB$^{AE}$</th>
<th>Ln MCR$^B$</th>
<th>Ln [p]AB</th>
<th>[p]AB$^B$</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>2370</td>
<td>1080</td>
<td>-11.8263</td>
<td>7.1624</td>
<td>-4.6639</td>
<td>0.94</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>2510</td>
<td>1190</td>
<td>-12.1804</td>
<td>''</td>
<td>-4.9108</td>
<td>0.66</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>2720</td>
<td>1150</td>
<td>-11.7691</td>
<td>''</td>
<td>-4.6067</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>2380</td>
<td>1250</td>
<td>-11.2786</td>
<td>''</td>
<td>-4.1162</td>
<td>1.63</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>1860</td>
<td>850</td>
<td>-11.2285</td>
<td>''</td>
<td>-4.0661</td>
<td>1.71</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>1490</td>
<td>840</td>
<td>-11.1075</td>
<td>''</td>
<td>-3.9451</td>
<td>1.90</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>M</td>
<td>2090</td>
<td>1100</td>
<td>-11.4341</td>
<td>''</td>
<td>-4.2717</td>
<td>1.40</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>M</td>
<td>2000</td>
<td>1150</td>
<td>-11.6731</td>
<td>''</td>
<td>-4.5107</td>
<td>1.10</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>M</td>
<td>2480</td>
<td>1510</td>
<td>-10.8233</td>
<td>''</td>
<td>-3.6609</td>
<td>2.57</td>
<td></td>
</tr>
</tbody>
</table>

**Mean ± SE**  2210 ±130  1125 ±70  -4.3177 ±0.1398  1.33 (1.01–1.76)%

Antilog mean (95% confidence limits)§

<table>
<thead>
<tr>
<th></th>
<th>10</th>
<th>F</th>
<th>1670</th>
<th>1020</th>
<th>-11.8470</th>
<th>7.1778</th>
<th>-4.6692</th>
<th>0.94</th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td>F</td>
<td>1830</td>
<td>1160</td>
<td>-12.3339</td>
<td>''</td>
<td>-5.1561</td>
<td>0.58</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>F</td>
<td>1700</td>
<td>1170</td>
<td>-11.9577</td>
<td>''</td>
<td>-4.7799</td>
<td>0.84</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>F</td>
<td>2430</td>
<td>1470</td>
<td>-12.4964</td>
<td>''</td>
<td>-5.3186</td>
<td>0.49</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>F</td>
<td>2030</td>
<td>1110</td>
<td>-12.0491</td>
<td>''</td>
<td>-4.3713</td>
<td>0.77</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>F</td>
<td>2470</td>
<td>1340</td>
<td>-11.8359</td>
<td>''</td>
<td>-4.6581</td>
<td>0.95</td>
<td></td>
</tr>
</tbody>
</table>

**Mean ± SE**  2020 ±140  1210 ±70  -4.9089 ±0.1107  0.74 (0.59–0.92)%

§ 95% confidence limits appear in parentheses.

* MCR$^A$ in liters/day per m$^2$.
‡ Ln MCR$^B$ is obtained from data in Longcope, Layne, and Tait (7) and stands for mean Ln of MCR$^B$ in liters/day per m$^2$.
§ 95% confidence limits appear in parentheses.

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and females for each steroid, but in both sexes the distribution coefficient is significantly greater \( (P < 0.01) \) for androstenedione than for testosterone.

**DISCUSSION**

The question as to whether there is any significant conversion of the circulating androgens, \( \Delta^4 \)-androstenedione and testosterone, to the estrogens, estrone and 17\( \beta \)-estradiol, has long been of interest. Studies in the past, notably those of Leach, Maddock, Tokuyama, Paulsen, and Nelson (10); West, Damast, Sarro, and Pearson (9); and Givner, Bauld, Hale, Vogi, and Nilsen (23) have shown that there is a rise in the urinary excretion of estrogens after the administration of testosterone propionate to males and nonpregnant females. This suggested that testosterone could be converted to estrogens but did not rule out a stimulatory effect of testosterone, or a metabolite, on estrogen synthesis. French, Baggett, Van Wyk, Talbert, Hubbard, Johnston, and Weaver (24) were not able to demonstrate any conversion of radioactive testosterone to the urinary metabolites of estradiol in individuals with feminizing testes. Since there is a defect in the peripheral utilization of testosterone in these individuals (24), this failure to note conversion is not, perhaps, applicable to normal individuals. Preedy, however, in several normal males, was also unable to demonstrate this conversion (25). This has not been the experience of others, and several groups have reported that radioactivity is recoverable in the urinary metabolites of the estrogens after the administration of radioactive testosterone (26-30) and \( \Delta^4 \)-androstenedione (11). This, then, is evidence that there is conversion of these androgens to estrogens, but it is not conclusive evidence that these conversions contribute significantly to the circulating pool of free estrogens. It has been shown, directly for the interconversion between adrostenedione and testosterone (2, 4) and indirectly for the interconversion of estrone and estradiol (7, 8), that the conversion of a precursor steroid to the urinary metabolite of a product steroid does not necessarily contribute to the blood pool of free product steroid.

Our results, however, obtained by infusing the radioactive precursor androgen and measuring the radioactivity in the free product estrogen show that the circulating free androgens are converted to circulating free estrogens. The fraction of androgen so converted is not large, about 1% of the androstenedione entering the blood \textit{de novo} is converted to estrogen, and about 0.4% of the testosterone in males and less than 0.2% in females is converted to estradiol.

* * *

**TABLE IV**

[\( \text{[P]}_{\text{BE}} \) Values for Testosterone Conversion to Estradiol]

<table>
<thead>
<tr>
<th>Subject</th>
<th>Sex</th>
<th>MCR(^T )</th>
<th>MCR(^T )</th>
<th>Ln( \frac{C_{\text{BE}}}{\text{MCR}^T} )</th>
<th>Ln MCR(^E )</th>
<th>Ln [( \text{[P]}_{\text{BE}} )]</th>
<th>( \text{[P]}_{\text{BE}} )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>liters/day</td>
<td>liters/day</td>
<td>per m(^2)</td>
<td>liters/day</td>
<td>per m(^2)</td>
<td>%</td>
</tr>
<tr>
<td>1</td>
<td>M</td>
<td>940</td>
<td>430</td>
<td>-12.1843</td>
<td>6.8773</td>
<td>-5.3070</td>
<td>0.50</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>1010</td>
<td>480</td>
<td>-12.3900</td>
<td>&quot;</td>
<td>-5.5177</td>
<td>0.40</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>850</td>
<td>360</td>
<td>-12.6125</td>
<td>&quot;</td>
<td>-5.7352</td>
<td>0.32</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>790</td>
<td>360</td>
<td>-12.2637</td>
<td>&quot;</td>
<td>-5.3864</td>
<td>0.46</td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>560</td>
<td>290</td>
<td>-12.3972</td>
<td>&quot;</td>
<td>-5.5199</td>
<td>0.40</td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>1000</td>
<td>560</td>
<td>-12.4938</td>
<td>&quot;</td>
<td>-5.6165</td>
<td>0.36</td>
</tr>
<tr>
<td>7</td>
<td>M</td>
<td>1180</td>
<td>650</td>
<td>-12.9153</td>
<td>&quot;</td>
<td>-6.0380</td>
<td>0.24</td>
</tr>
<tr>
<td>8</td>
<td>M</td>
<td>1100</td>
<td>580</td>
<td>-12.3546</td>
<td>&quot;</td>
<td>-5.4773</td>
<td>0.42</td>
</tr>
</tbody>
</table>

Mean ± SE  930 ±70  465 ±45  -5.5741 ±0.0808  0.38 (0.32-0.45)%

Antilog mean (95% confidence limits)

\( ^* \) MCR\(^T \) in liters/day per m\(^2\).

\( ^\dagger \) Ln MCR\(^E \) is obtained from data in Longcope, Layne, and Tait (7) and stands for mean Ln MCR\(^E \) in liters/day per m\(^2\).
The free estrogens that we measure are converted from androgens in the tissues and then appear in the blood before irreversible metabolism. Our data do not, however, indicate in what tissues the conversions occur. Human fetal liver (31), human mammary carcinoma (32), and rat liver and kidney (33) have all been shown capable of aromatizing androgens. It is also possible that the conversions represent aromatization of circulating androgens by adrenal or gonadal tissue. The data of MacDonald, Rombaut, and Siiteri (11) who found the fraction of radioactivity appearing in the urinary metabolites of estrogens after administration of radioactive androstenedione to be similar in normal, adrenalectomized, and castrated individuals makes this possibility less likely.

It has been shown that the placenta (34) aromatizes androstenedione somewhat more readily than testosterone, and it has been suggested that the aromatization of testosterone by placental microsomes occurs preferentially via androstenedione and 19-OH androstenedione (35). Our data also suggest that androstenedione is aromatized more readily than testosterone. However, the fact that circulating androstenedione is converted to circulating estradiol to a lesser degree than is testosterone suggests that, as regards measurements in the peripheral blood, the aromatization of testosterone does not occur preferentially via androstenedione. In all likelihood, the peripheral aromatization reaction occurs largely via the respective 19-OH intermediates and the 17-hydroxy group is preserved. The fact that we were not able to demonstrate conversion of androstenedione to estradiol or testosterone to estrone in many individuals is no indication that such reactions did not occur; it means only that these particular conversions, as measured in blood, occurred in most instances at a level below our current ability to detect. In actual fact, as shown in Fig. 1, the conversion of androstenedione to estradiol, and testosterone to estrone must occur, but, under the conditions used in our experiments, such conversions must be of a very low order.

For the conversion of androstenedione to estrone and testosterone to estradiol, whether measured directly as a conversion ratio, or calculated as a \([\rho]_{BB}^{PRE-PRO}\) value, the mean values in males were higher than the respective mean values in females. It would thus appear from measurements in blood that males are capable of greater peripheral aromatization of androgens to estrogens than are females. Whether this is possibly due to greater enzyme activity or to differences in the concentrations of precursor and/or product in blood cannot be delineated further from our data.

One of the important aspects of a \([\rho]_{BB}\) value is that it can be used to estimate the amount of product formed from precursor if the blood production rate of precursor and product are known. As defined by Horton and Tait (2) and used by them and others (2, 19, 36, 37), the blood production rate \((P_s)\) is defined as that amount of a steroid entering the blood, *de novo*, per unit time. The amount of product formed from precursor will then be the blood production rate of precursor multiplied by the \([\rho]_{BB}\) value for precursor conversion to product. We did not directly measure the blood production rates for the androgens and estrogens in our study, but we can obtain an estimate of the androgen contribution to estrogen production from the mean data of Baird, Horton, Longcope, and Tait (36) for androgen and estrogen production rates, which were obtained by

---

### Table V

**Mean CR\(_{BB}^{PRE-PRO}\) and \([\rho]_{BB}^{PRE-PRO}\) Values for the Interconversions of Androstenedione and Testosterone**

<table>
<thead>
<tr>
<th>Number</th>
<th>Sex</th>
<th>Precursor</th>
<th>Product</th>
<th>CR(_{BB}^{PRE-PRO})</th>
<th>([\rho]_{BB}^{PRE-PRO})</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>Male</td>
<td>Androstenedione</td>
<td>Testosterone</td>
<td>0.12 ± 0.01‡</td>
<td>4.5 (3.7-5.5)%§</td>
</tr>
<tr>
<td>6</td>
<td>Female</td>
<td>Androstenedione</td>
<td>Testosterone</td>
<td>0.10 ± 0.01</td>
<td>2.2 (1.7-2.9)%</td>
</tr>
<tr>
<td>8</td>
<td>Male</td>
<td>Testosterone</td>
<td>Androstenedione</td>
<td>0.034 ± 0.003</td>
<td>8.2 (6.2-11.0)%</td>
</tr>
<tr>
<td>6</td>
<td>Female</td>
<td>Testosterone</td>
<td>Androstenedione</td>
<td>0.027 ± 0.002</td>
<td>12.0 (9.7-15.0)%</td>
</tr>
</tbody>
</table>

* Calculated from Ln CR\(_{BB}^{PRE-PRO}\) (liters/day per m\(^3\)) plus Ln MCR\(_{PRO}\) (liters/day per m\(^3\)).
† Mean ± SE.
§ Antilog of the mean (95% confidence limits).

---

### Table VI

**Distribution Coefficients between Red Cells and Plasma for Precursor Radioactivity**

<table>
<thead>
<tr>
<th>No. of subjects</th>
<th>Sex</th>
<th>Precursor</th>
<th>Distribution coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>Male</td>
<td>Androstenedione</td>
<td>0.156 ± 0.042</td>
</tr>
<tr>
<td>3</td>
<td>Female</td>
<td>Androstenedione</td>
<td>0.171 ± 0.026</td>
</tr>
<tr>
<td>3</td>
<td>Male</td>
<td>Testosterone</td>
<td>0.026 ± 0.012</td>
</tr>
<tr>
<td>6</td>
<td>Female</td>
<td>Testosterone</td>
<td>0.009 ± 0.006</td>
</tr>
</tbody>
</table>

* Mean ± SE.
several groups in studies on individuals comparable with our subjects.

In this manner, we estimate that in males at least half the estradiol entering the blood, de novo, arises as a result of conversion of testosterone to estradiol. Thus, even though the $[\rho]_{\alpha}^{\text{estr}}$ is between 0.3 and 0.45% in the male, so much more testosterone (about 7 mg/day) is produced as compared with estradiol (about 40 µg/day) that the contribution of testosterone (about 20–30 µg/day) is highly significant. Our estimate that at least 50% of the estradiol arises from testosterone is in the same range as the earlier and more indirect estimates of Leach et al. (10) and Epstein, Raheja, Prow, and Morse (28).

On the other hand, in normal, nonpregnant females, testosterone (200–300 µg/day) and estradiol (100–300 µg/day) are produced in somewhat similar amounts for a large part of the cycle, and $[\rho]_{\alpha}^{\text{estr}}$ is only 0.16%, so that the contribution of testosterone to estradiol is insignificant.

In both males and females, the production of androstenedione is similar (about 3 mg/day) and is greater than the estimated $P_\alpha$ of estrone (about 125-150 µg/day). Therefore, even though the conversions of androstenedione to estrone are only about 1.5% in males and 0.7% in females, these contributions (about 30–50 µg/day) would appear to make up at least 20–30% of the estrone entering the blood per day de novo in both sexes.

The contribution of the androgens to the circulating estrogens, estrone and estradiol, in the male and estrone in the female would thus appear to be a highly significant source of these estrogens. The entire blood production rate of the estrogens, however, does not appear to result from these conversions. Whether the sources of the rest of the blood production rates are other precursors or direct secretion of the estrogens cannot be said from these data alone. However, since other precursors are unlikely (36, 37), it would appear that in males and females there is direct secretion of both estrone and estradiol.

Recently, Brenner, Hutchinson, Siteri, and MacDonald (38) reported that in normal males the entire production rate of estrone and estradiol could be accounted for by conversion from androstenedione and testosterone. This is at variance with our conclusions noted above, and the disagreement possibly is due to differences in calculating production rates and interconversions of the estrogens, since Brenner et al. (38) used a combined blood and urinary approach (36). Thus, they were equating an androgen blood production rate determined at a specific time of day with an estrogen production rate calculated from the integrated specific activity of urinary metabolites excreted over several days. In addition, the conversions of androstenedione to estradiol, 0.8%, and testosterone to estrone, 0.2%, as calculated from isotopic ratios of urinary metabolites, appear to be far higher than these particular conversions measured using the free steroid radioactivity in blood (0.0–0.1% for each). Thus, the use of data obtained from urinary metabolites may have slightly overestimated the contribution of the circulating androgens to the circulating estrogens.

Our mean values for the metabolic clearance rates of androstenedione and testosterone were similar to those reported previously by Bardin and Lipsett (19) and Southren, Gordon, Tochimoto, Pinzon, Lane, and Stypulkowski (39). We found no difference for MCR* between males and females, and in both sexes MCR* was greater than MCR$. We also found, however, that MCR$ was significantly greater in males than females. These differences in the clearance rates of MCR* and MCR$, and the greater MCR$ in males than females were probably a reflection in large part of the greater plasma globulin binding of testosterone in females as compared with males, and in both sexes the greater plasma globulin binding of testosterone as compared with androstenedione (40, 41). Similarly, the distribution coefficients between red cells and plasma for androstenedione and testosterone probably were another indication of the greater binding of testosterone, as compared with androstenedione, to a plasma globulin (42, 43). Testosterone which is strongly bound to plasma globulins has a lower distribution coefficient than androstenedione which is weakly bound to plasma globulins. It has also been suggested for cortisol (44) and for estradiol in females (7) that a similarly low distribution coefficient is secondary to the plasma globulin binding of these steroids.

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Also, our CR and [$\Delta$] values for the interconversions of androstenedione and testosterone were similar to those reported by others (19, 45). The [$\Delta$] values in both males and females were greater than the respective CR values, in keeping with the previous suggestions (36, 37) that the oxidative pathway is the more favored one, so far as these conversions are concerned.

ACKNOWLEDGMENTS

We wish to acknowledge the expert technical assistance of Mrs. Sue Balestra. Dr. James F. Tait gave invaluable help throughout the course of the study. Dr. Herbert Teagher, Boston University, gave invaluable assistance in certain of the statistical aspects of the study. The pipylate purification step was carried out with the kind assistance of Dr. David T. Baird.

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