IN VIVO INTERCONVERSION FACTORS BETWEEN OESTRONE AND 17\(\beta\)-OESTRADIOL IN RAT TISSUES \(\varphi_{TT}\)

By

R. De Hertogh*, E. Ekka and I. Vanderheyden

ABSTRACT

A method allowing of an approach to the \emph{in vivo} study of oestrone \(\Leftrightarrow\) 17\(\beta\)-oestradiol interconversion factors in peripheral tissues \(\varphi_{TT}\) is described. The mathematical treatment is based on the same parameters as used for the determination of blood interconversion factors \(\varphi_{BB}\), considering the tissue as a two-compartment open system with »penetration« of labelled hormones occurring in both compartments at different rates, depending on the type of hormone infused into the circulating blood.

Rat liver displays a complete interconversion \(\varphi_{TT} = 1\) between oestrone and 17\(\beta\)-oestradiol. The adrenals display partial interconversion, since a mean \(\varphi_{TT}\)

\[
\left(\text{i. e. } \sqrt{\frac{OE_1OE_2}{\varphi_{TT}^2} \times \frac{OE_2OE_1}{\varphi_{TT}^2}}\right)
\]

is found to be 0.12 to 0.14. Muscle and adipose tissue do not show any significant interconversion.

The experimental limitations of this method are discussed, and the application to other pairs of compounds is commented upon in view of the concept of prehormone.

Some peripheral tissues are capable of interconverting oestrone and 17\(\beta\)-oestradiol \emph{in vitro} (Ryan & Engel 1953). Interconversion occurs \emph{in vivo} as well, in as far as the whole subject is concerned, as reflected by blood measurements, leading to the determination of a »blood interconversion factor« \(\varphi_{BB}\) (Gurpide et al. 1963; Longcope et al. 1968; De Hertogh et al. 1970).

* Chercheur qualifié du Fonds National Belge de la Recherche Scientifique.
In vivo interconversion at the tissue level is an interesting feature to investigate, because of its possible relationship with hormonal activation or inactivation at the target site. For instance the prostate is likely to convert testosterone to 5α-dihydrotestosterone which in turn exerts a physiological activity (Bruchovsky & Wilson 1968; Baulieu et al. 1968). Conversion of oestrone to 17β-oestradiol would result in hormonal activation. Conversion at the tissue level is a particular aspect of the concept of »prehormone« as described by Baird et al. (1968). As far as hormonal activity is concerned, in vivo interconversion at the tissue level might be involved in a transhydrogenation system, as previously put forward by Talalay & Williams-Ashman (1960).

Long-term infusion of labelled hormone would allow equilibrium to be established between endogenous (unlabelled) and exogenous (labelled) metabolites, in the blood and tissues (Pearlman et al. 1966). Hence, the metabolite pattern at equilibrium will eventually reflect the physiological situation, resulting from blood production of the compound investigated (either oestrone or 17β-oestradiol).

By studying both compounds separately (or simultaneously with different isotopes), the difference in the patterns of labelled metabolites will reflect the result of incomplete interconversion between the compounds. Tissue accumulation of metabolites will be secondary to uptake factors (hence also to the composition of blood metabolites), and to intra-tissue metabolism. In view of these data an approach can be worked out to assess separately intra-tissue metabolism and in particular, intra-tissue interconversion.

**MATERIALS AND METHODS**

Groups of adult female Wistar-R rats were submitted to 3 h-intravenous infusions either with [6,7-3H]oestrone or with [6,7-3H]17β-oestradiol as described previously (De Hertogh et al. 1971).

Equilibrium was thus achieved and the tissue radioactivity was shown to be independent of the infusion rates in the liver, adrenals, adipose tissue and muscle. Results from either experiment could then be compared on a physiological basis.

The groups were previously defined as I, II, III, according to the histological picture of the uterus and vagina (De Hertogh et al. 1971).

The infusion method and extraction procedures have already been described as well as the experimental results in terms of »concentration index« (i.e. concentration in pg/g divided by the rate of infusion in ng/h) (De Hertogh et al. 1971).

**Calculation of the interconversion factor in tissue (QT) **

General mathematical treatment. Let us consider a two-compartment open system (Fig. 1), in which »secretion« (s₁ and s₂) occurs in both compartments. »Secretion« here is defined as direct entry of hormone into the compartment, from sources external to the system (in μmoles/min). The pool in each compartment (1 and 2) is subjected to
Two-compartment open system, in which »secretion« (s) and »penetration« of labelled hormone (π) occur in both compartments (1 and 2). See text for comments.

some renewal which is characterized by the »turn-over time« (t1 and t2) i.e. the time required for the replacement of that fraction of the pool present in one unit of the volume of distribution of the compartment (in min/ml).

Hence, at equilibrium t1 = \frac{b1}{p1} \quad (1)

where b1 is the concentration in compartment 1 at equilibrium (in μmoles/ml)

p1 is the »production rate« in compartment 1, i.e. secretion rate in compartment 1 increased by that fraction of the secretion rate in compartment 2 which is converted to compartment 1 (see below).

Similarly t2 = \frac{b2}{p2} \quad (2)

The transfer rates between compartments (r12 and r21 in μmoles/min) and the excretion rates from either compartment (e1 and e2 in μmoles/min) are constant at equilibrium.

The »transfer« or »interconversion factor« between compartments 1 and 2 (σ12) is defined as the ratio of the transfer rate from compartment 1 to compartment 2 and the total rate of hormone leaving compartment 1 (transfer rate and excretion rate).

\sigma^{12} = \frac{r^{12}}{r^{12} + e^1} \quad (3)

Inversely \sigma^{21} = \frac{r^{21}}{r^{21} + e^2} \quad (4)

Hence \quad p^1 = s^1 + s^2\sigma^{21} \quad (5)
\quad p^2 = s^2 + s^1\sigma^{12} \quad (6)
Replacing (5) and (6) in (1) and (2) respectively:

\[
t_1^1 = \frac{b^1}{s^1 + s^2 q^{21}}
\]

\[
t_1^2 = \frac{b^2}{s^2 + s^1 q^{12}}
\]

By introducing labelled hormones in the system at a constant rate (\(\pi^1\) and \(\pi^2\) in \(\mu\text{Ci/min}\)), the concentrations of labelled hormones in each compartment at equilibrium will be \(B_1\) and \(B_2\) (in \(\mu\text{Ci/ml}\)). \(\beta^1\) and \(\beta^2\) will be the specific activities in each compartment.

The turnover of labelled hormone is assumed to be similar to unlabelled compound. Hence interconversion factors (relations (3) and (4)) are independent of specific activities and it can be shown that:

\[
t_1^1 = \frac{B^1}{\pi^1 + \pi^2 q^{21}} \quad \text{and} \quad t_1^2 = \frac{B^2}{\pi^2 + \pi^1 q^{12}}
\]  

(9 and 9')

**Application to tissue interconversion factors** \(q_{TT}\) **between oestrone and 17\(\beta\)-oestadiol**

When [6,7-\(\text{H}\)]oestrone or [6,7-\(\text{H}\)]17\(\beta\)-oestadiol is infused intravenously, overall interconversion occurs. This can be measured in blood \(q_{BB}\) as described elsewhere (De Hertogh et al. 1970).

\[
q_{BB} = \frac{MCR_P \cdot OE_2 \cdot B_P \cdot OE_2}{MCR_P \cdot OE_1 \cdot B_P \cdot OE_1} \quad \text{and} \quad q_{BB} = \frac{MCR_P \cdot OE_1 \cdot B'_P \cdot OE_1}{MCR_P \cdot OE_2 \cdot B'_P \cdot OE_2}
\]

(10 and 10')

Where \(MCR_P\) are the metabolic clearance rates (Tait & Burnstein 1964).

\(B_P\) and \(B'_P\), \(OE_1\) and \(OE_2\) are the concentrations of [6,7-\(\text{H}\)]-oestrone and [6,7-\(\text{H}\)]17\(\beta\)-oestadiol in each type of infusion, respectively.

Hence, whatever the infused hormone, both compounds are in circulation. Tissues will then be subject to penetration by both [6,7-\(\text{H}\)]oestrone and [6,7-\(\text{H}\)]17\(\beta\)-oestadiol. The relative concentration of [6,7-\(\text{H}\)]oestrone will however be higher in [6,7-\(\text{H}\)]-oestrone infusion, and vice-versa.

Hence, in [6,7-\(\text{H}\)]oestrone infusion, »penetration rate« of both compounds from the blood into tissue may be defined as \(\pi' OE_1\) and \(\pi' OE_2\). In [6,7-\(\text{H}\)] 17\(\beta\)-oestadiol infusion, these rates will be \(\pi' OE_1\) and \(\pi' OE_2\).

Hence according to relations (9) and (10):

\[
t_{TT} = \frac{B_T OE_1}{\pi'_{OE_1} + \pi'_{OE_2} q_{TT}} = \frac{B'_T OE_1}{\pi'_{OE_1} + \pi'_{OE_2} q_{TT}}
\]

(11)

420
\[
\begin{align*}
\frac{O_{E_2}}{\ell_T} &= \frac{B_T}{O_{E_2}} \frac{O_{E_2}}{\pi_T + \pi_{E_1}} \frac{O_{E_1}O_{E_2}}{\ell_T} = \frac{B_T}{O_{E_2}} \frac{O_{E_2}}{\pi_T + \pi_{E_1}} \frac{O_{E_1}O_{E_2}}{\ell_T} \\
\end{align*}
\]

where \(B_T^{O_{E_1}}\) and \(B_T^{O_{E_2}}\), \(B_T^{O_{E_1}}\) and \(B_T^{O_{E_2}}\) are the concentrations in the tissue at equilibrium of \([6,7-^3\text{H}]\text{oestrone}\) or \([6,7-^3\text{H}]\text{17\beta-oestradiol}\), in each type of infusion respectively.

Relations (11) and (12) can be solved to give:

\[
\begin{align*}
\frac{O_{E_1}O_{E_2}}{\ell_T} &= \frac{B_T^{O_{E_2}}}{O_{E_2}} \frac{O_{E_2}}{\pi_T - \pi_{E_1}} \\
\frac{O_{E_2}O_{E_1}}{\ell_T} &= \frac{B_T^{O_{E_1}}}{O_{E_1}} \frac{O_{E_1}}{\pi_T - \pi_{E_2}}
\end{align*}
\]

Three possibilities can be considered:

1) \(\frac{\pi_{E_1}}{\pi_T} = \frac{O_{E_1}}{B_T^{O_{E_1}}} \) or, \(\frac{\pi_{E_2}}{\pi_T} = \frac{O_{E_2}}{B_T^{O_{E_2}}}\) (15 and 16)

in which case the concentration in the tissue is directly proportional to the penetration rate in each compartment.

Then, relations (13) and (14) simplify to:

\[
\begin{align*}
O_{E_2}O_{E_1} \ell_T &= 0 \\
O_{E_1}O_{E_2} \ell_T &= 0
\end{align*}
\]

\(\pi_T\) is not known. However, when tissue uptake is proportional to the concentration in the plasma as in the liver, adrenals, muscle and adipose tissue (De Hertogh et al. 1971), \(\pi_T\) will be some function of the plasma concentration. Hence:
Similarly:

\[
\frac{O_E_2O_E_1}{\theta_{TT}} = 0 \text{ when } \frac{B_T}{B_P} = \frac{O_E_1}{O_E_1} = \text{constant}
\]

\[
\text{or } \frac{O_E_1}{B_P} = \frac{O_E_1}{B_P} = \text{constant}
\]

Remark: The significance of »f« may be interpreted as follows: If \(\pi_{BT}\) is a fraction of \(\pi_{BB}\), the infusion rate into the blood, \(\pi_{BT} = \pi_{BB} \theta_{BT}\) where \(\theta_{BT}\) is the »transfer factor« from blood to tissue.

In \([6,7-3H]\)oestrone infusion for instance:

\[
\pi_{BB} = \text{the rate of infusion in blood } = B_P O_E_1 \times MCR_P O_E_1
\]

\[
\pi_{BT} = (B_P O_E_1 \times MCR_P O_E_1) \theta_{BT}
\]

\[
= f_1 B_P O_E_1
\]

where \(f_1 = MCR_B O_E_1 \times \theta_{BT} = \text{tissue virtual clearance rate for } O_E_1
\]

\(\text{in ml of plasma/min}\)

\[
\pi_{BT} = \pi_{BB} \times \theta_{BT}
\]

\[
\pi_{BB} = O_E_1 \theta_{BB} - \pi_{BB} \theta_{BB}
\]

\[
\text{where } \theta_{BB} = \text{the »blood interconversion factor« (i.e. overall interconversion factor, as reflected by blood (or plasma) measurements).}
\]

\[
O_E_1 \theta_{BT} = \text{the fraction of the infusion rate of } O_E_1 \text{ converted to blood } O_E_2, \text{ by the tissue under study.}
\]

\[
\theta_{BB} = \frac{O_E_1 \theta_{BB} \times O_E_1 \theta_{TT} \times O_E_2 \theta_{TB}}{}
\]

If the tissue is considered as a two-compartment open system, this fraction is indeed not to be considered as external to that system (see general mathematical treatment).
Hence, according to (10):

\[
\frac{\text{OE}_2}{\beta_T} = \text{MCR}_P \left( \frac{\text{OE}_2}{B'_P} - \frac{\text{OE}_2}{B_P} \right) \frac{\text{OE}_2}{\beta_T}
\]

\[
= f_2 \left( \frac{\text{OE}_2}{B'_P} - \frac{\text{OE}_2}{B_P} \right)
\]

(20)

where \( f_2 = \text{MCR}_P \times \frac{\text{OE}_2}{\beta_T} \) = tissue virtual clearance rate for \( \text{OE}_2 \)

(in ml of plasma/min)

\( B'_P \) is that fraction of plasma \([6,7-3H]17\beta\)-oestradiol concentration, due to

\([6,7-3H]\)oestrone conversion within

the tissue \((= \frac{\text{OE}_1}{B'_P} \times \frac{\text{OE}_1}{\beta_T} \times \frac{\text{OE}_1}{\beta_T})\).

Similarly, in \([6,7-3H]17\beta\)-oestradiol infusion:

\[
\frac{\text{OE}_2}{\beta_T} = f_2 \frac{\text{OE}_2}{B'_P}
\]

(21)

\[
\frac{\text{OE}_1}{\beta_T} = f_1 \left( \frac{\text{OE}_1}{B'_P} - \frac{\text{OE}_1}{B_P} \right)
\]

(22)

Equations (17) and (18) should then be appropriately written as follows:

\[
\frac{\text{OE}_2}{\beta_T} = 0 \text{ when } \frac{\text{OE}_1}{B_T} = \frac{\text{OE}_1}{B'_P} - \frac{\text{OE}_1}{B_P} \frac{\text{OE}_1}{\beta_T} \frac{\text{OE}_1}{\beta_T}
\]

(23)

\[
\frac{\text{OE}_1}{\beta_T} = 0 \text{ when } \frac{\text{OE}_2}{B'_T} = \frac{\text{OE}_2}{B'_P} - \frac{\text{OE}_2}{B_P} \frac{\text{OE}_2}{\beta_T} \frac{\text{OE}_2}{\beta_T}
\]

(24)

It is obvious that \( B'_P \) TP and \( B'_P \) TP will tend towards zero, if \( \beta_T \) values approach zero. Hence relations (17) and (18) are correct.

2) \[
\frac{\text{OE}_1}{\beta_T} > \frac{\text{OE}_1}{B'_T} \text{ or } \frac{\text{OE}_1}{\beta_T} < \frac{\text{OE}_1}{B'_T} \text{ then } \frac{\text{OE}_2}{\beta_T} > 0
\]

(25)

Similarly:

\[
\frac{\text{OE}_2}{\beta_T} > \frac{\text{OE}_2}{B'_T} \text{ or } \frac{\text{OE}_2}{\beta_T} < \frac{\text{OE}_2}{B'_T} \text{ then } \frac{\text{OE}_2}{\beta_T} > 0
\]

(26)
Remark: the qualitative significance of relations (25) and (26) will be reinforced if \( B_P' \) and \( B_P'' \) were corrected by \( B_P' OE_1 \) TP and \( B_P'' OE_2 \) TP respectively.

In this case, equations (13), (14) and (19) to (22) lead to calculation of the product \( OE_1 OE_2 \times OE_2 OE_1 \), as long as \( B_P' \) TP and \( B_P'' \) TP are neglected (if intra-tissue interconversion does not contribute significantly to the overall interconversion as measured by \( t_{BB} \)).

Individual value for \( \varphi_{TT} \) might be calculated only if either »f« values are known, or if \( t_{TT} \) values are known (from relations (11) and (12)).

3) In addition to circumstance 2, we may have:

\[
\frac{OE_1}{OE_2} = \frac{B_P' OE_1}{B_P'' OE_2} = \text{constant} \tag{27}
\]

then \( \varphi_{TT} OE_2 OE_1 \times \varphi_{TT} OE_2 OE_1 = 1 \)

Hence \( \varphi_{TT} OE_2 OE_1 \) and \( \varphi_{TT} OE_2 OE_1 = 1 \)

Here, the contribution of intra-tissue interconversion on the overall interconversion has no effects on the results, as the plasma concentration does not intervene in the computing of relation (27).

RESULTS

1) In vivo interconversion factors in the adipose tissue

Table 1 gives the tissue/plasma ratio obtained from concentrations at equilibrium of [6,7-\(^3\)H]oestrone and [6,7-\(^3\)H]17\(\beta\)-oestradiol in groups of rats infused either with [6,7-\(^3\)H]oestrone, or with [6,7-\(^3\)H]17\(\beta\)-oestradiol. The ratios are relatively constant for each hormone within each group, with either type of infusions. Hence, according to relations (17) and (18), it might be concluded that no in vivo interconversion occurs between oestrone and 17\(\beta\)-oestradiol in the adipose tissue.

2) In vivo interconversion factors in the muscle

Table 2 gives the tissue/plasma ratio in muscle. Although relatively constant values are obtained for each hormone, they are systematically higher when the opposite hormone is infused, which is in agreement with relations (25) and (26).

Hence, the product of \( \varphi_{TT} OE_1 OE_2 \times \varphi_{TT} OE_2 OE_1 \) may be calculated (relations
Table 1.
Ratio of [6,7-3H]oestrone (OE₁) or [6,7-3H]17β-oestradiol (OE₂) concentrations at equilibrium in adipose tissue and plasma of adult female rats infused for 3 h with [6,7-3H]-oestrone or [6,7-3H]17β-oestradiol.

<table>
<thead>
<tr>
<th>Infused hormones and groups</th>
<th>Tissue/plasma</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OE₁</td>
<td>OE₂</td>
<td></td>
</tr>
<tr>
<td>[6,7-3H]oestrone</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I (n = 9)</td>
<td>8.09</td>
<td>3.64</td>
<td></td>
</tr>
<tr>
<td>II (n = 5)</td>
<td>7.62</td>
<td>3.91</td>
<td></td>
</tr>
<tr>
<td>III (n = 7)</td>
<td>9.85</td>
<td>3.48</td>
<td></td>
</tr>
<tr>
<td>[6,7-3H]17β-oestradiol</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I (n = 9)</td>
<td>9.85</td>
<td>4.40</td>
<td></td>
</tr>
<tr>
<td>II (n = 6)</td>
<td>12.50</td>
<td>7.04</td>
<td></td>
</tr>
<tr>
<td>III (n = 7)</td>
<td>8.43</td>
<td>4.48</td>
<td></td>
</tr>
</tbody>
</table>

n = number of animals in the pool.

(13), (14) and (19) to (22).

Very low figures are obtained: Group I : $45 \times 10^{-6}$
Group II : 0
Group III: $7.2 \times 10^{-4}$

Hence, in vivo interconversion between oestrone and 17β-oestradiol in the muscle does not seem to be of great significance.

3) In vivo interconversion factors in the adrenals

Table 3 gives the tissue/plasma ratio in the adrenals. These ratios are systematically higher when the opposite hormone is infused, in agreement with relations (25) and (26).

Products of $\frac{OE₁OE₂}{TT} \times \frac{OE₂OE₁}{TT}$ are calculated from relations (13), (14) and (19) to (22), assuming that intra-adrenal interconversion would not appreciably influence the overall interconversion (and plasma concentrations of the products) because of the small size and the relatively low blood flow rate in the tissue (the maximal influence can be calculated as not exceeding 5 to 10% of the overall interconversion).

Group I : $1.95 \times 10^{-2}$
Group II : $1.95 \times 10^{-2}$
Group III: $1.88 \times 10^{-2}$
Table 2.
Ratio of [6,7-3H]oestrone (OE₁) or [6,7-3H]17β-oestradiol (OE₂) concentrations at equilibrium in muscle and plasma in groups of adult female rats infused for 3 h with [6,7-3H]oestrone or [6,7-3H]17β-oestradiol.

<table>
<thead>
<tr>
<th>Infused hormones and groups</th>
<th>Tissue/plasma</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OE₁</td>
<td>OE₂</td>
</tr>
<tr>
<td>[6,7-3H]oestrone</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I (n = 9)</td>
<td>1.09</td>
<td>1.21</td>
</tr>
<tr>
<td>II (n = 5)</td>
<td>1.00</td>
<td>1.09</td>
</tr>
<tr>
<td>III (n = 6)</td>
<td>1.17</td>
<td>1.40</td>
</tr>
<tr>
<td>[6,7-3H]17β-oestradiol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I (n = 9)</td>
<td>1.67</td>
<td>1.20</td>
</tr>
<tr>
<td>II (n = 6)</td>
<td>1.00</td>
<td>0.83</td>
</tr>
<tr>
<td>III (n = 7)</td>
<td>1.51</td>
<td>1.18</td>
</tr>
</tbody>
</table>

n = number of animals in the pool.

Table 3.
Ratio of [6,7-3H]oestrone (OE₁) or [6,7-3H]17β-oestradiol (OE₂) concentrations at equilibrium in the adrenals and plasma, in groups of adult female rats infused for 3 h with [6,7-3H]oestrone or [6,7-3H]17β-oestradiol.

<table>
<thead>
<tr>
<th>Infused hormones and groups</th>
<th>Tissue/plasma</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OE₁</td>
<td>OE₂</td>
</tr>
<tr>
<td>[6,7-3H]oestrone</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I (n = 9)</td>
<td>10.3</td>
<td>23.1</td>
</tr>
<tr>
<td>II (n = 5)</td>
<td>9.0</td>
<td>22.1</td>
</tr>
<tr>
<td>III (n = 7)</td>
<td>13.1</td>
<td>34.4</td>
</tr>
<tr>
<td>[6,7-3H]17β-oestradiol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I (n = 9)</td>
<td>25.7</td>
<td>12.0</td>
</tr>
<tr>
<td>II (n = 6)</td>
<td>20.6</td>
<td>10.4</td>
</tr>
<tr>
<td>III (n = 7)</td>
<td>17.8</td>
<td>11.2</td>
</tr>
</tbody>
</table>

n = number of animals in the pool.

A mean interconversion factor \(\sqrt{\frac{O_{E1}O_{E2}}{\varphi_{TT}}} \times \frac{O_{E2}O_{E1}}{\varphi_{TT}}\) of the order of 0.12 to 0.14 is thus to be considered.
4) *In vivo* interconversion factors in the liver

Table 4 gives the tissue/plasma ratio in the liver. Here the ratios are much higher when the opposite hormone is infused, showing considerable interconversion within the tissue (relations (25) and (26)).

Moreover, when considering the OE₁/OE₂ ratio in the tissue, with both types of infusions (Table 5), they are constant for each group in agreement with relation (27).

\[
\text{Hence } \frac{\text{OE}_1}{\text{OE}_2} \times \frac{\text{OE}_2}{\text{OE}_1} = 1 \text{ and interconversion, in either direction, might be considered as being complete in the liver.}
\]

**DISCUSSION**

*In vivo* interconversion between oestrone and 17β-oestradiol has been shown to be extensive in the liver \((\frac{\text{OE}_1}{\text{OE}_2} \text{ and } \frac{\text{OE}_2}{\text{OE}_1} = 1)\).

Further metabolism in the tissue will then have the same consequences for either hormone. Indeed, a very similar metabolism has been demonstrated for oestrone and 17β-oestradiol in the human, when urine metabolites are investigated (see Loraine 1958). Differences might be due to extra-hepatic metabolism,

**Table 4.**

Ratio of [6,7-³H]oestrone (OE₁) or [6,7-³H]17β-oestradiol (OE₂) concentrations at equilibrium in the liver and plasma in groups of adult female rats infused for 3 h with [6,7-³H]oestrone or [6,7-³H]17β-oestradiol.

<table>
<thead>
<tr>
<th>Infused hormones and groups</th>
<th>Tissue/plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OE₁</td>
</tr>
<tr>
<td>[6,7-³H]oestrone</td>
<td></td>
</tr>
<tr>
<td>I  (n = 7)</td>
<td>6.08</td>
</tr>
<tr>
<td>II (n = 4)</td>
<td>6.15</td>
</tr>
<tr>
<td>III (n = 6)</td>
<td>5.80</td>
</tr>
<tr>
<td>[6,7-³H]17β-oestradiol</td>
<td></td>
</tr>
<tr>
<td>I  (n = 5)</td>
<td>47.0</td>
</tr>
<tr>
<td>II (n = 4)</td>
<td>35.4</td>
</tr>
<tr>
<td>III (n = 5)</td>
<td>28.1</td>
</tr>
</tbody>
</table>

\(n = \text{number of animals in the pool.}\)
Table 5.
Ratio of [6,7-³H]oestrone (OE₁) and [6,7-³H]17β-oestradiol (OE₂) concentrations at equilibrium in tissues of adult female rats infused for 3 h with [6,7-³H]oestrone or [6,7-³H]17β-oestradiol.

<table>
<thead>
<tr>
<th>Infused hormones and groups</th>
<th>Adipose tissue</th>
<th>Muscle</th>
<th>Adrenals</th>
<th>Liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>[6,7-³H]oestrone I</td>
<td>29.4</td>
<td>11.7</td>
<td>5.81</td>
<td>12.5</td>
</tr>
<tr>
<td>II</td>
<td>28.6</td>
<td>13.3</td>
<td>6.10</td>
<td>11.0</td>
</tr>
<tr>
<td>III</td>
<td>26.3</td>
<td>11.3</td>
<td>5.10</td>
<td>8.0</td>
</tr>
<tr>
<td>[6,7-³H]17β-oestradiol I</td>
<td>0.87</td>
<td>0.23</td>
<td>0.39</td>
<td>14.3</td>
</tr>
<tr>
<td>II</td>
<td>0.50</td>
<td>0.25</td>
<td>0.40</td>
<td>10.5</td>
</tr>
<tr>
<td>III</td>
<td>0.41</td>
<td>0.29</td>
<td>0.37</td>
<td>9.5</td>
</tr>
</tbody>
</table>

which is of significant importance for these hormones in the rat (De Hertogh et al. 1970) and human (Longcope et al. 1968). The extended interconversion of oestrone and 17β-oestradiol in the liver can be compared with the rather limited interconversion as measured in blood

\[ \varrho_{BB}^{OE_1OE_2} = 8\% \text{, } \varrho_{BB}^{OE_2OE_1} = 14 \text{ to } 20\% \text{ in the rat} \] (De Hertogh et al. 1970). The uptake of oestrogen by the liver should thus involve a rather extended clearance by this organ, and a limited re-entry of the hormone into the blood. This again is in agreement with the elevated metabolic clearance rate of oestrone and 17β-oestradiol in the rat (De Hertogh et al. 1970).

In vivo interconversion between oestrone and 17β-oestradiol in the adrenals is not surprising because of the high metabolic activity of this organ. Whether this interconversion intervenes in a biosynthetic mechanism is not known at present. Hydrogen exchange between androgens and oestrogens has been shown to occur in the liver, placenta and ovary (Wenzel & Pollow 1968).

Adipose tissue and muscle do not show any significant activity, in as far as interconversion between oestrone and 17β-oestradiol is concerned.

One limitation of the procedure described in this paper is the need to perform two series of infusions, one with each compound of the interconverting pair.

The use of one series of infusions including both compounds, each labelled with a different isotope (¹⁴C-oestrone and ³H-oestradiol) would involve practical difficulties because of the 1000 fold difference in the respective specific
activity of $^3$H and $^{14}$C labelled compounds. Considerable overloading of the system would thus be required if both compounds were to be infused in comparable weights. On the other hand, the use of two series of infusions with both hormones labelled with tritium, as performed in the present experiments, would require investigating the constancy of $f$ values (relations (19) to (22)) from one series of experiments to the other. This was shown to be the case in the liver, adrenals, muscle and adipose (De Hertogh et al. 1971). In the uterus however, $f$ values are not constant throughout the range of $17\beta$-oestradiol levels in the plasma, as shown by the non-proportional uptake of radioactivity with increasing infusion rates (De Hertogh et al. 1971). Hence, in these tissues, where binding sites of limited capacity exist, infusion rates should remain in the $f$ level in order to keep $f$ values constant from one series of experiments to another. Difficulties in radioactive measurements of metabolites within small tissues would then arise, and larger pools would be required.

Nevertheless, in vivo interconversion of other pairs of compounds (androstenedione – testosterone, testosterone-dihydrotestosterone, progesterone-20α-hydroxyprogesterone, etc ...) can be studied by this method in appropriate tissues, and possibly lend support to the concept of prehormone, as described by Baird et al. (1968).

ACKNOWLEDGMENTS

The Fonds National Belge de la Recherche Scientifique, the Fonds de la Recherche Scientifique Médicale and the Fondation Médicale Reine Elisabeth are gratefully acknowledged for their support in the experimental work.

REFERENCES

Loraine J. A.: The clinical application of hormone assay, E. and S. Livingstone Ltd.,
Received on May 2nd 1970.