An improved model of the distribution and metabolism of progesterone and 20α-dihydroprogesterone in sheep

J. Y. F. Paterson, F. A. Harrison, E. L. Sheldrick and R. B. Heap


Summary. When [3H]progesterone is infused intravenously into ewes, blood 20α-dihydroprogesterone (20α-diHP) becomes labelled and the changes in [3H]20α-diHP activity with time are clearly related to that of [3H]progesterone. Concentrations of 20α-diHP in blood have now been estimated for these experiments. During the infusion, the specific radioactivity of 20α-diHP at steady state was only 53% of the specific radioactivity of progesterone, indicating that 20α-diHP was produced other than by C-20 reduction of secreted progesterone. The change in blood concentration of 20α-diHP during pregnancy in ewes suggests that the placenta is its source.

[3H]20α-dihydroprogesterone was infused into pregnant ewes and the specific radioactivity of 20α-diHP measured during and after infusion. Together with information from earlier experiments when [3H]progesterone was infused, there is now sufficient data to estimate, without constraint, the parameters of a four-pool model describing the distribution and metabolism of progesterone and 20α-dihydroprogesterone in sheep.

Introduction

Paterson, Bedford, Harrison & Heap (1976) described the specific radioactivity of progesterone and the concentration of [3H]20α-dihydroprogesterone (20α-diHP) in blood during and after infusion of [7α-3H]progesterone in sheep. On the basis of their observations they proposed a model containing two pools of progesterone and two pools of 20α-diHP with irreversible conversion of progesterone to dihydroprogesterone in both pools. The model contained more parameters than could be estimated uniquely from the available experimental information, and constraints were applied to obtain a solution.

We have now estimated the specific radioactivity of 20α-diHP during and after infusion of [1,2-3H]20α-dihydroprogesterone in sheep. In these experiments no [3H]progesterone could be detected and the earlier assumption that conversion of progesterone to 20α-diHP is irreversible is upheld.

We have also estimated blood concentrations of 20α-diHP; our earlier assumption that during infusion of [3H]progesterone the equilibrium specific radioactivities of progesterone and 20α-diHP are equal was not upheld. It was therefore necessary to modify the earlier model to include production of 20α-dihydroprogesterone from some source other than progesterone in circulating blood. The additional experimental information provides sufficient degrees of freedom to allow the estimation of the model parameters without imposition of constraints.

Materials and Methods

Animals and techniques

Sheep. Clun Forest and Clun Forest × Merino sheep were used. Details of the animals, their feeding and management have been described previously (Bedford, Harrison & Heap, 1972).
Infusion of 20α-dihydroprogesterone. [1,2-3H]20α-Hydroxypregn-4-en-3-one (sp. act. 45 Ci/mmol; Radiochemical Centre, Amersham, U.K.) was infused at constant rate of 1 µCi (7 ng)/min into a jugular vein for 3-5 h. The sampling procedure was identical to that described by Paterson et al. (1976).

Analysis of blood samples. The amounts of [1H]progesterone and of [3H]20α-dihyHP were measured using the method of Bedford et al. (1972). The concentrations of endogenous progesterone and of 20α-diHP in plasma were measured by radioimmunoassay and adjusted to blood concentrations using measured haematocrit. Progesterone was determined by method A of Heap, Gwyn, Laing & Walters (1973) using the antiserum BF 465 No. 6 (kindly supplied by Dr B. J. A. Furr) which was raised in a goat immunized against progesterone conjugated to bovine serum albumin through the 11-position. The main cross-reactants of the antiserum were progesterone (100%), 11α-hydroxyprogren-4-ene-3,20-dione (117%) and 5α-pregnane-3,20-dione (35%). In the concentration range 2 to 10 ng/ml the intra-assay variation was 16.3% and interassay variation 19.6%.

20α-Dihydroprogesterone was estimated by a procedure similar to that for progesterone, but petroleum ether was replaced by diethyl ether for the extraction of plasma. The antiserum used was P003 (Steranti Research Ltd, St Albans, Herts, U.K.), raised in rabbits against the bovine serum albumin conjugate of 20α-diHP 3-(O-carboxymethyl)-oxime. The cross-reactivity of the antiserum with different steroids was determined as the concentration of 20α-diHP divided by the concentration of other steroids which caused 50% displacement expressed as a percentage. The values obtained were 100% for 20α-diHP, 19.8% for 3β,20α-dihydroxyprog-5-ene (20α-dihydropregnenolone), 8.4% for progesterone, 1.0% for pregnenediol, 0.4% for 20β-diHP, 0.1% for 5α-dihydroprogesterone, and <0.1% for 17α-hydroxyprogesterone, pregnanetriol, 16α-hydroxyprogesterone, 17α-hydroxyprogrenolone, 5β-dihydroprogesterone, deoxycorticosterone, corticosterone, cortisol, pregnenolone, 17α,20α-dihydroprogesterone, testosterone, 3β,5β-pregnanolone, 3α,5α-pregnanolone, 3β,5α-pregnanolone and dehydroepiandrosterone (DHA). Over the concentration range 2 to 15.5 ng/ml the intra-assay variation was 11.1% and interassay variation was 18.1%.

The recoveries of progesterone and 20α-diHP from plasma were 78.4 ± 1.1% and 79.6 ± 3.0% respectively, and values for concentrations were corrected accordingly. The sensitivities of the assays for progesterone and 20α-diHP were 28 and 20 pg/tube respectively (calculated from 2 × s.d. below zero point).

20α-Dihydroprogrenolone as a contaminant in 20α-diHP assays. Diethyl ether extracts (5 ml) of 2 ml plasma were concentrated and applied to a thin-layer chromatograph plate (silica gel 60 F-254, Merck). Using methylene chloride:diethyl ether (5:2 v/v) at 4°C the chromatogram was run twice over a 17-cm distance. Authentic steroids (Medicinal Research Council Steroid Reference Collection, Hampstead, London) were run in adjacent lanes on the same t.l.c. plate.

Consecutive 2-3 mm bands of the lanes containing plasma extracts were removed, eluted with ethanol, evaporated and their immunoreactivity measured using antiserum P003. Lanes containing authentic steroids were sprayed with glacial acetic acid containing 1% anisaldehyde and 2% sulphuric acid and heated briefly at 70°C. The Rf values of 20α-diHP and 20α-dihydroprogrenolone were 0.46 and 0.32 respectively. The Rf values for other steroids tested in the same chromatographic system were 5β-pregnane-3α, 17α, 20α-triol, <0.01; 5β-pregnane-3α, 20α-diol, 0.17; 17α-hydroxyprogesterone, 0.42; 20β-hydroxyprogren-4-en-3-one, 0.45; deoxycorticosterone, 0.48; DHA, 0.51; 5β-pregnane-3α-ol-20-one, 0.51; 5α-pregnane-3β-ol-20-one, 0.52; oestradiol-17β, 0.53; pregnenolone, 0.55; 5β-pregnane-3β-ol-20-one, 0.59; oestradiol-17α, 0.62; progesterone, 0.72; 5β-pregnane-3,20-dione, 0.85; 5α-pregnene-3,20-dione, 0.87.

Mathematical methods

The model is shown in Text-fig. 1: Q1 and Q2 are the two pools of progesterone and Q4 and Q3 the pools of 20α-diHP; Q1 defines pool size (µg) and αi its specific radioactivity (µCi/µg); si is the
Progesterone and 20α-dihydroprogesterone in sheep

**Text fig. 1.** A four-pool model of progesterone and 20α-dihydroprogesterone (20α-diHP) distribution and metabolism in sheep. $Q_1$ and $Q_2$ are the two pools of progesterone and $Q_4$ and $Q_3$ the pools of 20α-diHP; $\alpha_1$ and $\alpha_2$, and $\alpha_4$ and $\alpha_3$ are the specific activities of progesterone and 20α-diHP, respectively; $s_1$ is the secretion rate of progesterone and $s_2$ the rate of production of 20α-diHP from sources other than secreted progesterone. The proportion of $Q_i$ transferred per unit time to $Q_j$ is represented by the rate constant $k_{ij}$.

The secretion rate of progesterone and $s_2$ the rate of production of 20α-diHP from sources other than secreted progesterone. Later $i_1$ and $i_2$ will be used to represent rates of infusion of [3H]progesterone and [3H]20α-dihydroprogesterone, respectively.

The proportion of $Q_i$ transferred per unit time to $Q_j$ is represented by the rate constant $k_{ij}$. The sum of all rate constants for $Q_i$ is $k_{ii}$. The rate of change of $Q_i$, $dQ_i/dt$, is represented by $Q'_i$ and the model is then described by the set of linear differential equations.

\[
\begin{align*}
Q'_1 &= s_1 - k_{11}Q_1 + k_{12}Q_2 \\
Q'_2 &= k_{21}Q_1 - k_{22}Q_2 \\
Q'_3 &= k_{32}Q_2 - k_{33}Q_3 + k_{34}Q_4 \\
Q'_4 &= s_2 + k_{41}Q_1 + k_{43}Q_3 - k_{44}Q_4
\end{align*}
\]

These equations can be solved readily by Laplace transformation, and in the steady state the pool sizes are

\[
\begin{align*}
Q_1 &= k_{22}s_1/\lambda_1\lambda_2 \\
Q_2 &= k_{21}s_1/\lambda_1\lambda_2 \\
Q_3 &= ([k_{44}k_{32}k_{21} + k_{41}k_{34}k_{22}]s_1 + [\lambda_1\lambda_2k_{34}]s_2)/\lambda_1\lambda_2\lambda_3\lambda_4 \\
Q_4 &= ([k_{43}k_{32}k_{21} + k_{41}k_{33}k_{22}]s_1 + [\lambda_1\lambda_2k_{33}]s_2)/\lambda_1\lambda_2\lambda_3\lambda_4
\end{align*}
\]

where

\[
\begin{align*}
\lambda_1\lambda_2 &= (k_{11} + k_{22} ± [(k_{11} + k_{22})^2 - 4(k_{11}k_{22} - k_{21}k_{12})]^{1/2})/2 \\
\lambda_3\lambda_4 &= (k_{33} + k_{44} ± [(k_{33} + k_{44})^2 - 4(k_{33}k_{44} - k_{43}k_{34})]^{1/2})/2
\end{align*}
\]
The differential equations can also be written in terms of specific radioactivities during and after infusion of labelled progesterone (i₁) or 20α-dihydroprogesterone (i₂). The solutions for the accessible pools of progesterone (Q₁) and 20α-diHP (Q₄) are as follows.

**Infusion of $[^{3}H]$progesterone**

\[
\alpha_1(t) = \frac{i_1}{s_1} \left( 1 - \frac{\lambda_2(k_{22} - \lambda_1)}{k_{22} + \lambda_2} e^{-\lambda_2 t} - \frac{\lambda_1(k_{22} - \lambda_2)}{k_{22}(\lambda_1 - \lambda_2)} e^{-\lambda_1 t} \right)
\]

\[
\alpha_4(t) = \frac{(k_{43}k_{32}k_{21} + k_{41}k_{22}k_{33})s_1}{(k_{43}k_{32}k_{21} + k_{41}k_{22}k_{33})s_1 + (\lambda_4\lambda_3\lambda_2)s_2} \left( 1 - \frac{\lambda_2\lambda_3\lambda_4(k_{41}k_{22} - \lambda_1)(k_{33} - \lambda_1) + k_{43}k_{32}k_{21})e^{-\lambda_2 t}}{(k_{43}k_{32}k_{21} + k_{41}k_{22}k_{33})(\lambda_2 - \lambda_1)(\lambda_3 - \lambda_1)(\lambda_4 - \lambda_1)} \right) - \frac{\lambda_1\lambda_2\lambda_3\lambda_4(k_{41}k_{22} - \lambda_2)(k_{33} - \lambda_2) + k_{43}k_{32}k_{21})e^{-\lambda_2 t}}{(k_{43}k_{32}k_{21} + k_{41}k_{22}k_{33})(\lambda_1 - \lambda_2)(\lambda_3 - \lambda_2)(\lambda_4 - \lambda_2)} - \frac{\lambda_1\lambda_2\lambda_3\lambda_4(k_{41}k_{22} - \lambda_3)(k_{33} - \lambda_3) + k_{43}k_{32}k_{21})e^{-\lambda_3 t}}{(k_{43}k_{32}k_{21} + k_{41}k_{22}k_{33})(\lambda_1 - \lambda_3)(\lambda_2 - \lambda_3)(\lambda_4 - \lambda_3)} - \frac{\lambda_1\lambda_2\lambda_3\lambda_4(k_{41}k_{22} - \lambda_4)(k_{33} - \lambda_4) + k_{43}k_{32}k_{21})e^{-\lambda_4 t}}{(k_{43}k_{32}k_{21} + k_{41}k_{22}k_{33})(\lambda_1 - \lambda_4)(\lambda_2 - \lambda_4)(\lambda_3 - \lambda_4)} \right)
\]

**Infusion of $[^{3}H]$20α-dihydroprogesterone**

\[
\alpha_4(t) = \frac{\lambda_1\lambda_2\lambda_3\lambda_4\lambda_5}{(k_{43}k_{32}k_{21} + k_{41}k_{22}k_{33})s_1 + (\lambda_1\lambda_2\lambda_3)s_2} \left( 1 - \frac{\lambda_4(k_{33} - \lambda_3)}{k_{33}(\lambda_4 - \lambda_3)} e^{-\lambda_4 t} - \frac{\lambda_3(k_{33} - \lambda_4)}{k_{33}(\lambda_3 - \lambda_4)} e^{-\lambda_3 t} \right)
\]

When equilibrium specific radioactivity has been attained and the infusion is then discontinued, the appropriate function is equilibrium specific activity minus the expression shown above, but with $t'$, time after the ending of infusion.

**Estimation of model parameters.** There are 12 parameters to be estimated, the 10 rate constants $k_{01}$-$k_{34}$ and the 2 entry rates $s_1$ and $s_2$. These were used to derive, iteratively, the exponential coefficients and constants for $\alpha_1$ and $\alpha_4$ shown above. From these, and the experimental values of $(t)$ and $(t')$, the predicted values of specific activity $\hat{a}_{ij}$ and $\hat{a}_{4j}$ were obtained. The experimentally measured specific activities were lognormally distributed, and so the sum of squares to be minimized was

\[
S = \sum_{j=1}^{J=N} [\ln(a_j/\hat{a}_j)]^2
\]

The subroutines E04HBF and E04JBF from the Mk 7 NAG library (Numerical Algorithms Group, Banbury Road, Oxford) were used for the minimization procedure. The initial values for the parameters were taken from Paterson et al. (1976).

**Results**

**Plasma concentrations of endogenous 20α-diHP and progesterone**

The immunoreactivity of plasma extracts after thin-layer chromatography and elution of consecutive strips of silica gel is shown in Text-fig. 2. The immunoreactivity measured by
Text-fig. 2. Identification and quantification of 20α-dihydroprogesterone (20α-diHP) and 20α-dihydropregnenolone in plasma extracts by thin-layer chromatography on silica gel, elution and reaction with 20α-dihydroprogesterone antiserum P003. Sample 1 is from sheep C355 at 134 days p.c. and Sample 2 from sheep F286 at 112 days p.c. The hatched bars indicate the location of authentic 3β,20α-dihydroxy pregn-5-ene ($R_f$ 0.32) and 20α-hydroxy pregn-4-en-3-one ($R_f$ 0.46); progesterone has an $R_f$ of 0.72.

antiserum P003 was mainly associated with two bands, chromatographically identical with authentic 20α-dihydroprogesterone and 20α-dihydropregnenolone.

Nine plasma samples from 4 sheep were examined in this way and there was a highly significant correlation ($r = 0.984$, 7 d.f.) between the concentrations of 20α-diHP ($x$; 5.6 to 34.2 ng) and 20α-dihydropregnenolone ($y$; 7.8 to 47.5 ng). The regression equation was $y = 2.1 (± 2.3) + 1.39 (± 0.09) x$. Since the intercept was not significantly different from zero, the slope was calculated through the origin (1.466 ± 0.037). Plasma extracts of 20α-diHP, not purified by t.l.c., therefore contain 100/1.68 = 60% 20α-dihydropregnenolone. The cross-reactivity of 20α-dihydropregnenolone with P003 antiserum is only 20% that of 20α-diHP and the immunoreactivity of such unpurified extracts is (0.4 × 1.0 + 0.6 × 0.2)/(0.4 × 1.0) = 1.3 times that for the true content of 20α-diHP.

Progesterone and 20α-dihydroprogesterone concentrations were measured in 43 plasma samples from non-pregnant (oestrous cycle) and pregnant ewes. The progesterone concentration was 2.3 ± 0.7 ng/ml in non-pregnant ewes and it increased in pregnancy to 2.4 ± 0.7 ng/ml (Days 1-50), 13.1 ± 1.7 ng/ml (Days 51-100), 8.3 ± 1.5 ng/ml (Days 101-125) and 7.1 ng/ml (Day 126 to term). The 20α-diHP concentration (corrected) was 1.9 ± 0.2 ng/ml in non-pregnant animals and 2.8 ± 0.2, 10.1 ± 1.3, 14.6 ± 2.3 and 11.6 ± 1.4 ng/ml in pregnancy (Days 1-50, 51-100, 101-125, 126-term, respectively).

Specific activity of 20α-diHP during and after infusion of [1,2-3H]20α-dihydroprogesterone

[3H]20α-dihydroprogesterone was infused into a jugular vein in 6 experiments on 5 pregnant ewes between Days 117 and 138 post coitum. Arterial blood concentrations of 20α-diHP and progesterone are shown in Table 1 together with the fitted time curves for [3H]20α-diHP concentrations during and after the infusion. Since the between-experiment differences in blood [3H]20α-
Table 1. Arterial blood concentrations of progesterone and 20α-dihydroprogesterone (20α-diHP) and computed [3H]20α-dihydroprogesterone time curves during and after infusion of [3H]20α-dihydroprogesterone, standardized to an infusion rate of 1 µCi/min

<table>
<thead>
<tr>
<th>Sheep</th>
<th>Days pregnant</th>
<th>20α-diHP (ng/ml)</th>
<th>Progesterone (ng/ml)</th>
<th>E (µCi/l)</th>
<th>A</th>
<th>B (min⁻¹)</th>
<th>α (min⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rennet</td>
<td>117</td>
<td>7.7</td>
<td>2.8</td>
<td>0.329</td>
<td>0.619</td>
<td>0.381</td>
<td>0.534</td>
</tr>
<tr>
<td>Rennet</td>
<td>138</td>
<td>6.1</td>
<td>3.8</td>
<td>0.350</td>
<td>0.542</td>
<td>0.458</td>
<td>1.006</td>
</tr>
<tr>
<td>Whim</td>
<td>120</td>
<td>3.4</td>
<td>4.5</td>
<td>0.386</td>
<td>0.563</td>
<td>0.437</td>
<td>0.370</td>
</tr>
<tr>
<td>Warble</td>
<td>120</td>
<td>5.5</td>
<td>8.1</td>
<td>0.492</td>
<td>0.683</td>
<td>0.317</td>
<td>0.800</td>
</tr>
<tr>
<td>Witch</td>
<td>138</td>
<td>2.8</td>
<td>5.0</td>
<td>0.387</td>
<td>0.690</td>
<td>0.310</td>
<td>0.708</td>
</tr>
<tr>
<td>Treacle</td>
<td>136</td>
<td>3.9</td>
<td>2.6</td>
<td>0.367</td>
<td>0.746</td>
<td>0.254</td>
<td>2.074</td>
</tr>
<tr>
<td>Pooled</td>
<td></td>
<td>4.9</td>
<td>4.5</td>
<td>0.380</td>
<td>0.640</td>
<td>0.360</td>
<td>0.785</td>
</tr>
</tbody>
</table>

E = equilibrium tracer concentration; A, B = fractional constants (A + B = 1); α, β = exponential coefficients.

* The exponential function fitted to blood [3H]20α-diHP is of the form:

\[ [3H]20α-diHP = E(1 - A e^{-\alpha t} - B e^{-\beta t}) \text{ µCi/l during infusion}, \]

and

\[ = (A e^{-\alpha t} + B e^{-\beta t}) \text{ µCi/l after infusion}. \]

diHP and [3H]progesterone concentrations appeared to be random, the standardized values, calculated as described by Paterson et al. (1976), were pooled (Table 2).

Table 2. Whole blood concentrations of [3H]progesterone and [3H]20α-dihydroprogesterone (20α-diHP) during and after infusions of labelled progesterone and 20α-diHP

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Mean ± s.e.m. conc. (µCi/l)</th>
<th>[3H]20α-diHP (1 µCi/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Progesterone</td>
<td>20α-diHP</td>
</tr>
<tr>
<td>145</td>
<td>0.302 ± 0.0138</td>
<td>0.158 ± 0.0114</td>
</tr>
<tr>
<td>175</td>
<td>0.316 ± 0.0179</td>
<td>0.175 ± 0.0243</td>
</tr>
<tr>
<td>205</td>
<td>0.302 ± 0.0145</td>
<td>0.154 ± 0.0128</td>
</tr>
<tr>
<td>(240 infusion stopped)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td>0.243 ± 0.0170</td>
<td>0.161 ± 0.0199</td>
</tr>
<tr>
<td>1</td>
<td>0.200 ± 0.0166</td>
<td>0.157 ± 0.0170</td>
</tr>
<tr>
<td>2</td>
<td>0.157 ± 0.0107</td>
<td>0.141 ± 0.0128</td>
</tr>
<tr>
<td>3</td>
<td>0.127 ± 0.0094</td>
<td>0.135 ± 0.0127</td>
</tr>
<tr>
<td>4</td>
<td>0.110 ± 0.0071</td>
<td>0.125 ± 0.0110</td>
</tr>
<tr>
<td>5</td>
<td>0.092 ± 0.0057</td>
<td>0.120 ± 0.0103</td>
</tr>
<tr>
<td>15</td>
<td>0.053 ± 0.0050</td>
<td>0.099 ± 0.0147</td>
</tr>
<tr>
<td>30</td>
<td>0.035 ± 0.0037</td>
<td>0.077 ± 0.0150</td>
</tr>
<tr>
<td>45</td>
<td>0.029 ± 0.0040</td>
<td>0.055 ± 0.0101</td>
</tr>
<tr>
<td>60</td>
<td>0.023 ± 0.0034</td>
<td>0.042 ± 0.0097</td>
</tr>
</tbody>
</table>

The concentrations for [3H]progesterone infusion are taken from Paterson et al. (1976) with n = 23. The concentrations for [3H]20α-diHP infusion are for the 6 experiments described in this paper.
The specific radioactivities of progesterone and 20α-diHP for infusion rates of 1 µCi/min, are shown in Text-fig. 3 together with the functions fitted in parameter estimation, which were

\[
\begin{align*}
\text{(a) } & \quad [{}^3\text{H}]\text{Prog} = 0.0660 \left( 1 - e^{-0.4373t} - 0.2407e^{-0.0202t} \right) \mu\text{Ci/µg} \\
\text{(b) } & \quad [{}^3\text{H}]20\alpha = 0.0348 \left( 1 - 0.9906e^{-0.4373t} + 5.9402e^{-0.0202t} + 0.5995e^{-0.7346t} - 6.549e^{-0.0194t} \right) \mu\text{Ci/µg} \\
\text{(c) } & \quad [{}^3\text{H}]20\alpha = 0.0774 \left( 1 - 0.6328e^{-0.7346t} - 0.3672e^{-0.0194t} \right) \mu\text{Ci/µg}
\end{align*}
\]

Equations (a) and (b) refer to the infusion of [3H]progesterone and (c) to the infusion of [3H]20α-diHP.

The estimate of the parameters and their standard errors of estimate are given in Table 3, and the pool sizes and fluxes for the two steroids derived from these parameters are shown in Text-fig. 4.

\[\text{Table 3. Parameters and standard errors of estimate for the distribution and metabolism of progesterone and 20α-dihydroprogesterone}\]

<table>
<thead>
<tr>
<th>Present work</th>
<th>Paterson et al. (1976)</th>
</tr>
</thead>
<tbody>
<tr>
<td>k_{01}</td>
<td>0.1214 ± 0.0129 min^{-1}</td>
</tr>
<tr>
<td>k_{21}</td>
<td>0.1823 ± 0.0248 min^{-1}</td>
</tr>
<tr>
<td>k_{41}</td>
<td>0.1276 ± 0.0161 min^{-1}</td>
</tr>
<tr>
<td>k_{02}</td>
<td>0.00797 ± 0.00059 min^{-1}</td>
</tr>
<tr>
<td>k_{12}</td>
<td>0.01365 ± 0.00288 min^{-1}</td>
</tr>
<tr>
<td>k_{32}</td>
<td>0.00466 ± 0.00102 min^{-1}</td>
</tr>
<tr>
<td>k_{03}</td>
<td>0.0081 ± 0.00053 min^{-1}</td>
</tr>
<tr>
<td>k_{43}</td>
<td>0.0221 ± 0.0037 min^{-1}</td>
</tr>
<tr>
<td>k_{04}</td>
<td>0.3801 ± 0.0672 min^{-1}</td>
</tr>
<tr>
<td>k_{34}</td>
<td>0.3437 ± 0.0731 min^{-1}</td>
</tr>
<tr>
<td>s_1</td>
<td>15.15 ± 0.60 µg/min (progesterone)</td>
</tr>
<tr>
<td>s_2</td>
<td>6.11 ± 0.45 µg/min (20α-diHP)</td>
</tr>
</tbody>
</table>
Text-fig. 4. Computed parameters of the four-pool model of progesterone and 20α-dihydroprogesterone distribution and metabolism in pregnant sheep. The values for pool size (µg) and for fluxes (µg/min) correspond to the parameters described in Text-fig. 1 and in the text.

The MCR of 20α-dihydroprogesterone (s2; µg/min/arterial blood concentration, µg/l) was 1.24 l/min compared with that of 3.36 l/min for progesterone.

Discussion

Bedford et al. (1972) observed a close correlation between the equilibrium concentrations in blood of [3H]progesterone and [3H]20α-dihydroprogesterone during infusion of [3H]progesterone. The slope of the regression line was indistinguishable from that observed by Short & Moore (1959) for the relation between endogenous progesterone and 20α-diHP concentrations in ovine blood. It was on this basis that we had earlier assumed that the 20α-dihydroprogesterone arose solely by reduction of secreted progesterone, and consequently the specific radioactivities of the two steroids were equal. We have now estimated the blood concentrations of 20α-hydroxypregnen-4-en-3-one and have found that, relative to progesterone, they were greater than expected. During infusion of [3H]-progesterone the equilibrium specific activity of [3H]20α-diHP is 53% that of progesterone and it is clear that there must be a source of 20α-diHP other than C-20 reduction of circulating progesterone. The results of the present study show that the metabolic clearance rate of 20α-diHP is 1.24 l/min compared with an estimated value of 3.36 l/min for progesterone, the latter value being similar to that reported previously (3.64 l/min; Bedford et al., 1972).

Pregn-5-ene-3β,20α-diol (20α-dihydropregnenolone) is also present in sheep plasma and is a contaminant in our 20α-diHP assay. Due allowance was made for this contamination and the concentrations of 20α-diHP in blood samples taken from Day 126 to term are similar to those reported by Short & Moore (1959) who used chromatographic separation and u.v. spectrophotometry, and by Elsner et al. (1980) who used a celite chromatographic separation and two radioimmunoassays with different antisera. Progesterone concentrations measured in our study were identical with those found by Short & Moore (1959) but at the lower end of the range of values recorded by Elsner.
et al. (1980). In the present study the concentrations of 20α-dihydroprogesterone and 20α-dihydro pregnenolone were closely correlated and it would appear likely that they originate from the same source.

Our observations refer mainly to late pregnancy in ewes (120 days p.c. or more) when placental secretion of progesterone is dominant. Ainsworth & Ryan (1967) showed that the ovine placenta in vitro could produce progesterone from pregnenolone, and also that it could metabolize progesterone to a variety of 20α, 20β and ring A reduction products. Pierrepont, Anderson, Turnbull & Griffiths (1973), in similar studies, have confirmed and extended these observations. Our observation that the blood concentration of 20α-diHP increases after Day 50 of pregnancy suggests that its source is the placenta since this is the time when placental secretion of progesterone becomes substantial (Ricketts & Flint, 1980) and that this is also the source of 20α-dihydroprogrenolone. The blood concentrations of this latter steroid are greater than those of 20α-diHP and it is therefore surprising that it has not been reported as a metabolite of pregnenolone or progesterone in in-vitro studies. It may be that in the whole animal the 20α-dihydroprogrenolone is secreted into blood flowing through the uterus, whereas when chopped placentomes are used the steroid is more readily exposed to enzyme systems which reduce it further.

The assumption by Paterson et al. (1976) that progesterone was the sole source of 20α-dihydroprogesterone was extended to the assumption that both steroids occupied the same volumes of distribution. From the observations of Bedford et al. (1974) it was assumed that 30% of the metabolism of each steroid could be accounted for by irreversible splanchnic extraction as represented by $k_{01}$ and $k_{04}$ (see Text-fig. 1). By the use of such constraints Paterson et al. (1976) were able to estimate values for the remaining parameters in the model. The observations described in the present paper have necessitated the inclusion in the model of a source of 20α-dihydroprogesterone other than C-20 reduction of secreted progesterone. At the same time, the observations on 20α-diHP specific activity during and after infusion of $^3$H$20α$-dihydroprogesterone provide sufficient extra degrees of freedom to enable us to determine the model parameters without making any assumptions or constraints.

During the infusion of $^3$H$20α$-dihydroprogesterone there was no observable $^3$H-labelling of progesterone, indicating that the reduction at C-20 is irreversible, which had been a tentative conclusion of Paterson et al. (1976). It follows that the changes resulting from application of the unconstrained estimation of parameters reported here must be confined largely to the 20α-diHP pools in the model. Paterson et al. (1976) had assumed that 30% of secreted progesterone was removed by splanchnic extraction (as represented by $k_{01}$) and this is the value estimated in the present paper. In the model of Paterson et al. (1976) 46% of progesterone was converted to 20α-diHP, and in the present work we calculate that there is 53% C-20 reduction of progesterone to 20α-diHP, predominantly from the smaller pool of progesterone.

The endogenous production of 20α-dihydroprogesterone represented as $s_2$ in the model (Text-fig. 1) is 40% of the secretion rate for progesterone (Text-fig. 4). This results in pool sizes for 20α-diHP similar to those estimated by Paterson et al. (1976). They had noted that their solution of the model was not particularly sensitive to variation of the exchange of 20α-diHP between pools 3 and 4. The present unconstrained estimation of parameters indicates that the interchange of 20α-diHP ($k_{34}$ and $k_{43}$) is more rapid than the exchange of progesterone ($k_{21}$ and $k_{12}$). It had been assumed by Paterson et al. (1976) that splanchnic extraction ($k_{04}$) of 20α-diHP accounted for 35% of its metabolism, and we have now estimated that this accounts for 78% of 20α-diHP removal. The latter value is about double that of progesterone, and the results imply 20α-diHP removal by some source other than the splanchnic organs.

In the analysis of tracer kinetic data it is a common practice to divide the pool sizes for a substance by its measured concentration in blood, and so express the pools as volumes of distribution. Paterson et al. (1976) determined that progesterone and 20α-diHP pools were equivalent to 8 and 70 litres blood steroid, respectively, and since the mean body weight of their sheep was 41 kg, it was concluded that both steroids must attain tissue concentrations considerably
greater than blood concentration. This could be achieved by binding to intracellular receptors, and through the high lipid solubility of progesterone. It is an assumption in tracer kinetic analysis that each pool has characteristic kinetic behaviour and in consequence each pool has a uniform specific activity. This cannot be strictly true for the large pools of progesterone and 20α-diHP, and any estimated parameters of distribution must then be approximate.

The assumption by Paterson et al. (1976) that the two steroids occupied the same volume of distribution has not been confirmed. Apparent pool volumes of progesterone are 10 and 69 litres (in terms of blood steroid) while the 20α-dihydropregesterone volumes are 5.6 and 73 litres. The probable inhomogeneity of the larger pools, discussed above, may make the apparent difference insignificant but this is unlikely to be so for the smaller pools. It seems likely that some tissues in rapid equilibrium with blood steroid contain much greater quantities of progesterone than of 20α-dihydropregesterone, and that an organ(s) with high blood flow (e.g. gravid uterus) is the site of rapid conversion of progesterone to 20α-dihydropregesterone.

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References


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