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

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

[^3H]20 α -dihydroprogesterone was infused into pregnant ewes and the specific radioactivity of 20 α -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 20 α -dihydroprogesterone in sheep.

Introduction

Paterson, Bedford, Harrison & Heap (1976) described the specific radioactivity of progesterone and the concentration of [^3H]20 α -dihydroprogesterone (20 α -diHP) in blood during and after infusion of [^3H]progesterone in sheep. On the basis of their observations they proposed a model containing two pools of progesterone and two pools of 20 α -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 20 α -diHP during and after infusion of [1,2- ^3H]20 α -dihydroprogesterone in sheep. In these experiments no [^3H]progesterone could be detected and the earlier assumption that conversion of progesterone to 20 α -diHP is irreversible is upheld.

We have also estimated blood concentrations of 20 α -diHP; our earlier assumption that during infusion of [^3H]progesterone the equilibrium specific radioactivities of progesterone and 20 α -diHP are equal was not upheld. It was therefore necessary to modify the earlier model to include production of 20 α -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 $\times$ Merino sheep were used. Details of the animals, their feeding and management have been described previously (Bedford, Harrison & Heap, 1972).

Infusion of 20 α -dihydroprogesterone. [1,2-³H]20 α -Hydroxypregn-4-en-3-one (sp. act. 45 Ci/mmol; Radiochemical Centre, Amersham, U.K.) was infused at constant rate of 1 μ Ci (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 [³H]progesterone and of [³H]20 α -diHP were measured using the method of Bedford *et al.* (1972). The concentrations of endogenous progesterone and of 20 α -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%), 11 α -hydroxypregn-4-ene-3,20-dione (117%) and 5 α -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%.

20 α -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 20 α -diHP 3-(O-carboxymethyl)-oxime. The cross-reactivity of the antiserum with different steroids was determined as the concentration of 20 α -diHP divided by the concentration of other steroids which caused 50% displacement expressed as a percentage. The values obtained were 100% for 20 α -diHP, 19.8% for 3 β ,20 α -dihydroxypregn-5-ene (20 α -dihydropregnenolone), 8.4% for progesterone, 1.0% for pregnanediol, 0.4% for 20 β -diHP, 0.1% for 5 α -dihydroprogesterone, and <0.1% for 17 α -hydroxyprogesterone, pregnanetriol, 16 α -hydroxyprogesterone, 17 α -hydroxypregnenolone, 5 β -dihydroprogesterone, deoxycorticosterone, corticosterone, cortisol, pregnenolone, 17 α ,20 α -dihydroprogesterone, testosterone, 3 β ,5 β -pregnanolone, 3 α ,5 α -pregnanolone, 3 β ,5 α -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 20 α -diHP from plasma were $78.4 \pm 1.1\%$ and $79.6 \pm 3.0\%$ respectively, and values for concentrations were corrected accordingly. The sensitivities of the assays for progesterone and 20 α -diHP were 28 and 20 pg/tube respectively (calculated from $2 \times$ s.d. below zero point).

20 α -Dihydropregnenolone as a contaminant in 20 α -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 (Medical Research Council Steroid Reference Collection, Hampstead, London) were run in adjacent lanes on the same t.l.c. place.

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 R_f values of 20 α -diHP and 20 α -dihydropregnenolone were 0.46 and 0.32 respectively. The R_f values for other steroids tested in the same chromatographic system were 5 β -pregnane-3 α ,17 α ,20 α -triol, <0.01; 5 β -pregnane-3 α ,20 α -diol, 0.17; Δ^4 -pregnene-3 β ,20 α -diol, 0.32; 5 β -pregnane-3 β ,20 α -diol, 0.35; 17 α -hydroxyprogesterone, 0.42; 20 β -hydroxypregn-4-en-3-one, 0.45; deoxycorticosterone, 0.48; DHA, 0.51; 5 β -pregnan-3 α -ol-20-one, 0.51; 5 α -pregnan-3 β -ol-20-one, 0.52; oestradiol-17 β , 0.53; pregnenolone, 0.55; 5 β -pregnan-3 β -ol-20-one, 0.59; oestradiol-17 α , 0.62; progesterone, 0.72; 5 β -pregnane-3,20-dione, 0.85; 5 α -pregnane-3,20-dione, 0.87.

Mathematical methods

The model is shown in Text-fig. 1: Q_1 and Q_2 are the two pools of progesterone and Q_4 and Q_3 the pools of 20 α -diHP; Q_1 defines pool size (μ g) and α_1 its specific radioactivity (μ Ci/ μ g); s_1 is the

Text-fig. 1. A four-pool model of progesterone and 20 α -dihydroprogesterone (20 α -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 20 α -diHP; α_1 and α_2 , and α_4 and α_3 are the specific activities of progesterone and 20 α -diHP, respectively; s_1 is the secretion rate of progesterone and s_2 the rate of production of 20 α -diHP from sources other than secreted progesterone. The proportion of Q_1 transferred per unit time to Q_3 is represented by the rate constant k_{ji} .

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

The proportion of Q_i transferred per unit time to Q_j is represented by the rate constant k_{ji} . 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.

$$\begin{aligned} Q_1' &= s_1 - k_{11}Q_1 + k_{12}Q_2 \\ Q_2' &= k_{21}Q_1 - k_{22}Q_2 \\ Q_3' &= k_{32}Q_2 - k_{33}Q_3 + k_{34}Q_4 \\ Q_4' &= s_2 + k_{41}Q_1 + k_{43}Q_3 - k_{44}Q_4 \end{aligned}$$

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

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

where

$$\begin{aligned} \lambda_1, \lambda_2 &= (k_{11} + k_{22} \pm [(k_{11} + k_{22})^2 - 4(k_{11}k_{22} - k_{21}k_{12})]^{1/2})/2 \\ \lambda_3, \lambda_4 &= (k_{33} + k_{44} \pm [(k_{33} + k_{44})^2 - 4(k_{33}k_{44} - k_{43}k_{34})]^{1/2})/2 \end{aligned}$$

The differential equations can also be written in terms of specific radioactivities during and after infusion of labelled progesterone (i_1) or 20α -dihydroprogesterone (i_2). The solutions for the accessible pools of progesterone (Q_1) and 20α -diHP (Q_4) are as follows.

Infusion of [3H]progesterone

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

$$\alpha_4(t) = \frac{(k_{43}k_{32}k_{21} + k_{41}k_{22}k_{33})i_1}{(k_{43}k_{32}k_{21} + k_{41}k_{22}k_{33})s_1 + (\lambda_1\lambda_2k_{33})s_2} \left\{ 1 - \frac{\lambda_2\lambda_3\lambda_4(k_{41}(k_{22} - \lambda_1)(k_{33} - \lambda_1) + k_{43}k_{32}k_{21})e^{-\lambda_1 t}}{(k_{43}k_{32}k_{21} + k_{41}k_{22}k_{33})(\lambda_2 - \lambda_1)(\lambda_3 - \lambda_1)(\lambda_4 - \lambda_1)} \right.$$

$$- \frac{\lambda_1\lambda_3\lambda_4(k_{41}(k_{22} - \lambda_2)(k_{33} - \lambda_2) + k_{43}k_{32}k_{21})e^{-\lambda_2 t}}{(k_{43}k_{32}k_{21} + k_{41}k_{22}k_{33})(\lambda_1 - \lambda_2)(\lambda_3 - \lambda_2)(\lambda_4 - \lambda_2)}$$

$$- \frac{\lambda_1\lambda_2\lambda_4(k_{41}(k_{22} - \lambda_3)(k_{33} - \lambda_3) + k_{43}k_{32}k_{21})e^{-\lambda_3 t}}{(k_{43}k_{32}k_{21} + k_{41}k_{22}k_{33})(\lambda_1 - \lambda_3)(\lambda_2 - \lambda_3)(\lambda_4 - \lambda_3)}$$

$$\left. - \frac{\lambda_1\lambda_2\lambda_3(k_{41}(k_{22} - \lambda_4)(k_{33} - \lambda_4) + k_{43}k_{32}k_{21})e^{-\lambda_4 t}}{(k_{43}k_{32}k_{21} + k_{41}k_{22}k_{33})(\lambda_1 - \lambda_4)(\lambda_2 - \lambda_4)(\lambda_3 - \lambda_4)} \right\}$$

Infusion of [3H]20 α -dihydroprogesterone

$$\alpha_4(t) = \frac{\lambda_1\lambda_2k_{33}i_2}{(k_{43}k_{32}k_{21} + k_{41}k_{22}k_{33})s_1 + (\lambda_1\lambda_2k_{33})s_2} \left\{ 1 - \frac{\lambda_4(k_{33} - \lambda_3)}{k_{33}(\lambda_4 - \lambda_3)} e^{-\lambda_3 t} - \frac{\lambda_3(k_{33} - \lambda_4)}{k_{33}(\lambda_3 - \lambda_4)} e^{-\lambda_4 t} \right\}$$

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 α_1 and α_4 shown above. From these, and the experimental values of (t) and (t') , the predicted values of specific activity $\hat{\alpha}_{1j}$ and $\hat{\alpha}_{4j}$ were obtained. The experimentally measured specific activities were lognormally distributed, and so the sum of squares to be minimized was

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

The subroutines EO4HBF and EO4JBF 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 20 α -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 20 α -dihydroprogesterone (20 α -diHP) and 20 α -dihydropregnenolone in plasma extracts by thin-layer chromatography on silica gel, elution and reaction with 20 α -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 3 β ,20 α -dihydroxypregn-5-ene (R_f 0.32) and 20 α -hydroxypregn-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 20 α -dihydroprogesterone and 20 α -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 20 α -diHP (x ; 5.6 to 34.2 ng) and 20 α -dihydropregnenolone (y ; 7.8 to 47.5 ng). The regression equation was $y = 2.1 (\pm 2.3) + 1.39 (\pm 0.09) x$. Since the intercept was not significantly different from zero, the slope was calculated through the origin (1.466 ± 0.037). Plasma extracts of 20 α -diHP, not purified by t.l.c., therefore contain $100/1.68 = 60\%$ 20 α -dihydropregnenolone. The cross-reactivity of 20 α -dihydropregnenolone with P003 antiserum is only 20% that of 20 α -diHP and the immunoreactivity of such unpurified extracts is $(0.4 \times 1.0 + 0.6 \times 0.2)/(0.4 \times 1.0) = 1.3$ times that for the true content of 20di-HP.

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

[3 H]20 α -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 20 α -diHP and progesterone are shown in Table 1 together with the fitted time curves for [3 H]20 α -diHP concentrations during and after the infusion. Since the between-experiment differences in blood [3 H]20 α -

Table 1. Arterial blood concentrations of progesterone and 20 α -dihydroprogesterone (20 α -diHP) and computed [3 H]20 α -dihydroprogesterone time curves during and after infusion of [3 H]20 α -dihydroprogesterone, standardized to an infusion rate of 1 μ Ci/min

Sheep	Days pregnant	20 α -diHP (ng/ml)	Progesterone (ng/ml)	[3 H]20 α -diHP*				
				E (μ Ci/l)	A	B	α (min^{-1})	β (min^{-1})
Rennet	117	7.7	2.8	0.329	0.619	0.381	0.534	0.0382
Rennet	138	6.1	3.8	0.350	0.542	0.458	1.006	0.0148
Whim	120	3.4	4.5	0.386	0.563	0.437	0.370	0.0142
Warble	120	5.5	8.1	0.492	0.683	0.317	0.800	0.0221
Witch	138	2.8	5.0	0.387	0.690	0.310	0.708	0.0259
Treacle	136	3.9	2.6	0.367	0.746	0.254	2.074	0.0167
Pooled		4.9	4.5	0.380	0.640	0.360	0.785	0.0206

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

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

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

$$\text{and} \quad = (A e^{-\alpha t} + B e^{-\beta t}) \mu\text{Ci/l after infusion.}$$

diHP and [3 H]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 [3 H]progesterone and [3 H]20 α -dihydroprogesterone (20 α -diHP) during and after infusions of labelled progesterone and 20 α -diHP

[3 H]progesterone (1 μ Ci/min)			[3 H]20 α -diHP (1 μ Ci/min)	
Time (min)	Mean $\pm$ s.e.m. conc. (μ Ci/l)		Time (min)	20 α -diHP conc. (μ Ci/l)
	Progesterone	20 α -diHP		
145	0.302 $\pm$ 0.0138	0.158 $\pm$ 0.0114	175	0.387 $\pm$ 0.0219
175	0.316 $\pm$ 0.0179	0.175 $\pm$ 0.0243	205	0.382 $\pm$ 0.0198
205	0.302 $\pm$ 0.0145	0.154 $\pm$ 0.0128	235	0.387 $\pm$ 0.0317
(210 infusion stopped)			(240 infusion stopped)	
0.5	0.243 $\pm$ 0.0170	0.161 $\pm$ 0.0199	1	0.242 $\pm$ 0.0336
1	0.200 $\pm$ 0.0166	0.157 $\pm$ 0.0170	2	0.179 $\pm$ 0.0234
2	0.157 $\pm$ 0.0107	0.141 $\pm$ 0.0128	3	0.160 $\pm$ 0.0192
3	0.127 $\pm$ 0.0094	0.135 $\pm$ 0.0127	4	0.147 $\pm$ 0.0151
4	0.110 $\pm$ 0.0071	0.125 $\pm$ 0.0110	5	0.149 $\pm$ 0.0161
5	0.092 $\pm$ 0.0057	0.120 $\pm$ 0.0103	10	0.117 $\pm$ 0.0233
15	0.053 $\pm$ 0.0050	0.099 $\pm$ 0.0147	15	0.089 $\pm$ 0.0113
30	0.035 $\pm$ 0.0037	0.077 $\pm$ 0.0150	30	0.069 $\pm$ 0.0101
45	0.029 $\pm$ 0.0040	0.055 $\pm$ 0.0101	45	0.057 $\pm$ 0.0104
60	0.023 $\pm$ 0.0034	0.042 $\pm$ 0.0097	60	0.050 $\pm$ 0.0090

The concentrations for [3 H]progesterone infusion are taken from Paterson *et al.* (1976) with $n = 23$. The concentrations for [3 H]20 α -diHP infusion are for the 6 experiments described in this paper.

The specific radioactivities of progesterone and 20 α -diHP for infusion rates of 1 μ Ci/min, are shown in Text-fig. 3 together with the functions fitted in parameter estimation, which were

Text-fig. 3. Measured blood specific activities of (a) [3 H]progesterone and (b) [3 H]20 α -dihydroprogesterone (20 α -diHP) during and after the infusion of [3 H]progesterone, and (c) of [3 H]20 α -diHP during and after the infusion of [3 H]20 α -diHP in pregnant sheep. The computed lines for the blood concentrations are given.

$$(a) \text{ } [^3\text{H}]\text{Prog} = 0.0660 (1.0 - 0.7593e^{-0.4373t} - 0.2407e^{-0.0202t}) \mu\text{Ci}/\mu\text{g}$$

$$(b) \text{ } [^3\text{H}]20\alpha = 0.0348 (1.0 - 0.9906e^{-0.4373t} + 5.9402e^{-0.2020t} + 0.5995e^{-0.7346t} - 6.5491e^{-0.0194t}) \mu\text{Ci}/\mu\text{g}$$

$$(c) \text{ } [^3\text{H}]20\alpha = 0.0774 (1.0 - 0.6328e^{-0.7346t} - 0.3672e^{-0.0194t}) \mu\text{Ci}/\mu\text{g}$$

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

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.

Table 3. Parameters and standard errors of estimate for the distribution and metabolism of progesterone and 20 α -dihydroprogesterone

	Present work	Paterson <i>et al.</i> (1976)
k_{01}	$0.1214 \pm 0.0129 \text{ min}^{-1}$	0.1228 min^{-1}
k_{21}	$0.1823 \pm 0.0248 \text{ min}^{-1}$	0.3783 min^{-1}
k_{41}	$0.1276 \pm 0.0161 \text{ min}^{-1}$	0.0823 min^{-1}
k_{02}	$0.00797 \pm 0.00059 \text{ min}^{-1}$	0.0110 min^{-1}
k_{12}	$0.01365 \pm 0.00288 \text{ min}^{-1}$	0.0185 min^{-1}
k_{32}	$0.00466 \pm 0.00102 \text{ min}^{-1}$	0.0124 min^{-1}
k_{03}	$0.0081 \pm 0.00053 \text{ min}^{-1}$	0.0263 min^{-1}
k_{43}	$0.0221 \pm 0.0037 \text{ min}^{-1}$	0.0185 min^{-1}
k_{04}	$0.3801 \pm 0.0672 \text{ min}^{-1}$	0.1288 min^{-1}
k_{34}	$0.3437 \pm 0.0731 \text{ min}^{-1}$	0.1938 min^{-1}
s_1	$15.15 \pm 0.60 \mu\text{g/min}$ (progesterone)	$18.3 \mu\text{g/min}$
s_2	$6.11 \pm 0.45 \mu\text{g/min}$ (20 α -diHP)	0

Text-fig. 4. Computed parameters of the four-pool model of progesterone and 20 α -dihydroprogesterone distribution and metabolism in pregnant sheep. The values for pool size (μg) and for fluxes ($\mu\text{g}/\text{min}$) correspond to the parameters described in Text-fig. 1 and in the text.

The MCR of 20 α -diHP (s_2 $\mu\text{g}/\text{min}/\text{arterial blood concentration, } \mu\text{g}/\text{l}$) 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]20 α -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 20 α -diHP concentrations in ovine blood. It was on this basis that we had earlier assumed that the 20 α -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 20 α -hydroxypregn-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]20 α -diHP is 53% that of progesterone and it is clear that there must be a source of 20 α -diHP other than C-20 reduction of circulating progesterone. The results of the present study show that the metabolic clearance rate of 20 α -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-3 β ,20 α -diol (20 α -dihydropregnenolone) is also present in sheep plasma and is a contaminant in our 20 α -diHP assay. Due allowance was made for this contamination and the concentrations of 20 α -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 20 α -dihydroprogesterone and 20 α -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 20 α , 20 β 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 20 α -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 20 α -dihydropregnenolone. The blood concentrations of this latter steroid are greater than those of 20 α -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 20 α -dihydropregnenolone 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 20 α -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 20 α -dihydroprogesterone other than C-20 reduction of secreted progesterone. At the same time, the observations on 20 α -diHP specific activity during and after infusion of [3 H]20 α -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]20 α -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 20 α -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 20 α -diHP, and in the present work we calculate that there is 53% C-20 reduction of progesterone to 20 α -diHP, predominantly from the smaller pool of progesterone.

The endogenous production of 20 α -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 20 α -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 20 α -diHP between pools 3 and 4. The present unconstrained estimation of parameters indicates that the interchange of 20 α -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 20 α -diHP accounted for 35% of its metabolism, and we have now estimated that this accounts for 78% of 20 α -diHP removal. The latter value is about double that of progesterone, and the results imply 20 α -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 20 α -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 -dihydroprogesterone 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 -dihydroprogesterone, and that an organ(s) with high blood flow (e.g. gravid uterus) is the site of rapid conversion of progesterone to 20α -dihydroprogesterone.

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