Progesterone and 20 α -dihydroprogesterone in sheep: a model of their distribution and metabolism

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Summary. The rates of metabolism of progesterone and 20 α -dihydroprogesterone (20 α -diHP) in sheep have been measured during and after the infusion of tracer amounts of [3 H]progesterone. There were significant differences in the blood concentration of [3 H]progesterone between experiments, but these were not attributable to the stage of gestation or to the difference between pregnant and non-pregnant animals. The mean ($\pm$ S.E.M.) metabolic clearance rate of progesterone was 3.277 ± 0.239 litres blood/min.

The simplest model of the distribution of progesterone was one containing two pools, $V_1[P]$ and $V_2[P]$, where $[P]$ is the blood concentration of progesterone, and in 23 experiments on 7 sheep the mean pool dimensions were $7.8[P]$ μ g and $70[P]$ μ g, respectively. This model was developed to include the formation of 20 α -diHP from progesterone. Progesterone appeared to be the major source of 20 α -diHP, though this did not seem to be an obligatory metabolite. The parameters obtained gave comparably low residual deviations for both labelled steroids and were consistent with other observations made on progesterone clearance.

Introduction

Studies on progesterone metabolism in the sheep have shown that the clearance rate of the hormone from blood is high, ranging from 2.7 litres/min in the oestrous cycle, 3.48 litres/min in pregnancy, and up to 5.02 litres/min in lactational anoestrus. The production rate is about 6 μ g/min when a CL is fully functional, and as high as 72 μ g/min towards the end of gestation when placental secretion is at its greatest (Bedford, Harrison & Heap, 1972). In further studies it was found that the efficiency of splanchnic extraction of progesterone was 92%, and that splanchnic clearance accounted for 27% of the metabolic clearance rate of progesterone (Bedford *et al.*, 1974).

Short & Rowell (1962) studied the kinetics of progesterone metabolism in sheep using a single injection of a large mass of [14 C]progesterone and their results indicated that the rate of progesterone metabolism did not change during the second half of gestation. Other studies in women resulted in the hypothesis that progesterone was distributed in the body within two theoretical pools and estimations were made of their volumes and the half-life of progesterone they contained (Little, Tait, Tait & Erlenmeyer, 1966).

A notable feature of progesterone metabolism in the sheep is the relatively high concentration of circulating 20 α -dihydroprogesterone (20 α -diHP) (Short & Moore, 1959). This is probably formed from progesterone (Bedford *et al.*, 1972) in extra-splanchnic organs such as the kidney (Bedford *et al.*, 1973). The post-infusion clearance of labelled progesterone and 20 α -diHP from blood allows the measurement through modelling of the rate constants for the conversion of progesterone to 20 α -diHP, as examined in the present paper.

Materials and Methods

Animals and techniques

Sheep. Welsh Mountain, Clun Forest and Merino $\times$ Welsh Mountain sheep were used. Details

of the animals, their feeding and management have been described previously (Bedford *et al.*, 1972).

Progesterone infusion. [7α - ^3H]Progesterone, sp. act. >8.5 Ci/mmol, was infused at a constant rate (approx. $1 \mu\text{Ci/min}$, <40 ng/min) into a jugular vein for 3.5 hr. Three or four blood samples of 10–20 ml were taken from the contralateral carotid artery or jugular vein at 15–20 min intervals during the last hour of infusion (between 14.00 and 17.00 hours to minimize possible effects of any diurnal variation).

After the constant infusion of labelled progesterone was stopped, a series of blood samples (7 ml each) was taken 0.5, 1, 2, 3, 4 and 5 min later, followed by further samples (12 ml each) at 15, 30, 45 and 60 min. All samples were immediately placed in chilled vessels, laked with distilled water and stored at -15°C .

Further details of the infusion procedure are given by Bedford *et al.* (1972).

Analysis of blood samples. The concentrations of radioactive progesterone and 20α -diHP were measured according to the technique of Bedford *et al.* (1972). The specific activity of progesterone was calculated from the endogenous blood progesterone concentration determined by a competitive protein-binding technique described elsewhere (Bedford *et al.*, 1972).

Mathematical methods

Exponential curve fitting. It will be shown that the specific radioactivity of progesterone in blood during and after infusion of labelled progesterone can be fitted by a sum of two exponential terms. The simplest model of the distribution of progesterone which would be described by such a function is one, containing two pools, whose general form is:

where $[P]$ is the concentration of progesterone in blood, the sizes of the two pools are represented by $V_1[P]$ and $V_2[P]$, and their specific activities by a_1 and a_2 , during infusion of labelled progesterone at $Q \mu\text{Ci/min}$ and at a secretion rate of $s \mu\text{g/min}$; k_{ij} is the rate constant for the transfer of progesterone from pool j to pool i ; k_{0i} represents irreversible catabolism in pool i .

The rates of change of the specific activities are given by the differential equations:

$$V_1[P] \frac{da_1}{dt} = Q - (k_{01} + k_{21}) V_1[P] a_1 + k_{12} V_2[P] a_2 \quad (1)$$

$$V_2[P] \frac{da_2}{dt} = k_{21} V_1[P] a_1 - (k_{02} + k_{12}) V_2[P] a_2 \quad (2)$$

During infusion of labelled progesterone, the solutions of these equations are:

$$a_1(t) = \frac{(k_{02} + k_{12}) Q}{\alpha_1 \alpha_2 V_1[P]} \left\{ 1 - \frac{\alpha_1 \alpha_2 - \alpha_2 (k_{02} + k_{12})}{(\alpha_1 - \alpha_2) (k_{02} + k_{12})} e^{-\alpha_1 t} + \frac{\alpha_1 \alpha_2 - \alpha_1 (k_{02} + k_{12})}{(\alpha_1 - \alpha_2) (k_{02} + k_{12})} e^{-\alpha_2 t} \right\} \quad (3)$$

$$a_2(t) = \frac{(k_{02} + k_{12}) Q}{\alpha_1 \alpha_2 V_1[P]} \left\{ 1 + \frac{\alpha_2}{(\alpha_1 - \alpha_2)} e^{-\alpha_1 t} - \frac{\alpha_1}{(\alpha_1 - \alpha_2)} e^{-\alpha_2 t} \right\} \quad (4)$$

where t is time (min) from the beginning of infusion and

$$\alpha = \{(k_{01} + k_{02} + k_{12} + k_{21}) \pm \sqrt{(k_{01} + k_{02} + k_{12} + k_{21})^2 - 4(k_{01} k_{02} + k_{01} k_{12} + k_{02} k_{21})}\} / 2 \quad (5)$$

If the infusion of labelled progesterone is sufficiently long to establish the equilibrium specific

activity $Q/(k_{02} + k_{12})/(\alpha_1 \alpha_2 V_1[P])$, then when infusion is stopped the specific activities are given by:

$$a_1(t') = \frac{(k_{02} + k_{12})Q}{\alpha_1 \alpha_2 V_1[P]} \left\{ \frac{\alpha_1 \alpha_2 - \alpha_2(k_{02} + k_{12})}{(\alpha_1 - \alpha_2)(k_{02} + k_{12})} e^{-\alpha_1 t'} - \frac{\alpha_1 \alpha_2 - \alpha_1(k_{02} + k_{12})}{(\alpha_1 - \alpha_2)(k_{02} + k_{12})} e^{-\alpha_2 t'} \right\} \quad (6)$$

$$a_2(t') = \frac{(k_{02} + k_{12})Q}{\alpha_1 \alpha_2 V_1[P]} \left\{ -\frac{\alpha_2}{(\alpha_1 - \alpha_2)} e^{-\alpha_1 t'} + \frac{\alpha_1}{(\alpha_1 - \alpha_2)} e^{-\alpha_2 t'} \right\} \quad (7)$$

where t' is time (min) from the end of infusion and α is as defined in Equation 5.

These equations contain six parameters, four rate constants and two pool sizes. If only one specific activity is measured, e.g. $a_1(t)$ and $a_1(t')$, then only five parameters can be determined uniquely; the sixth parameter can only be estimated if the model is constrained in some way.

It can be seen that the equilibrium specific activity and the exponential constants and coefficients are common to Equation 3 and Equation 6 for a_1 . A continuous exponential function was fitted using Equations 3, 5 and 6 to the measured values of progesterone specific activity in blood, $a_1(t)$ and $a_1(t')$. The optimum values for the parameters were estimated using the simplex method of Nelder & Mead (1965).

20 α -Dihydroprogesterone. The blood concentration of labelled 20 α -diHP was also measured and was found to be related to the blood concentration of labelled progesterone (Bedford *et al.*, 1973). To investigate this relationship it was postulated that progesterone in both pools could be converted to 20 α -diHP. A four-pool model containing two pools each of progesterone and two of 20 α -diHP was examined. The simplest solution was to assume that in both progesterone pools the same proportion was converted to 20 α -diHP which implies that progesterone and 20 α -diHP occupy the same distribution volumes. V_i , k_{ij} and $[P]$ have been defined above, and $[D]$ is the blood concentration of 20 α -diHP.

This model is described by four differential equations:

$$V_1[P] \frac{da_1}{dt} = Q - (k_{01} + k_{21} + k_{41}) V_1[P] a_1 + k_{12} V_2[P] a_2 + k_{14} V_1[D] a_4 \quad (8)$$

$$V_2[P] \frac{da_2}{dt} = k_{21} V_1[P] a_1 - (k_{02} + k_{12} + k_{32}) V_2[P] a_2 + k_{23} V_2[D] a_3 \quad (9)$$

$$V_2[D] \frac{da_3}{dt} = k_{32} V_2[P] a_2 - (k_{03} + k_{23} + k_{43}) V_2[D] a_3 + k_{34} V_1[D] a_4 \quad (10)$$

$$V_1[D] \frac{da_4}{dt} = k_{41} V_1[P] a_1 + k_{43} V_2[D] a_3 - (k_{04} + k_{14} + k_{34}) V_1[D] a_4 \quad (11)$$

These equations were solved numerically using arbitrary values of parameters by the Runge-Kutta-Merson method (Gear, 1971). About 1 min of computer time was required for each solution for a 3.5 hr infusion, and 1 hr post-infusion. It was not practicable to link this method to a parameter-optimization procedure which might require several hundred iterations. Accordingly, the parameters were adjusted systematically to minimize the sum of squares of differences between measured and computed values of labelled 20 α -diHP concentrations.

Results

Blood progesterone and [³H]progesterone concentrations during and after intravenous infusion of [³H]progesterone

[³H]Progesterone was infused at about 1.0 µCi/min into a jugular vein for 3.5 hr. Blood samples were taken from the contralateral carotid artery or jugular vein during the last hour of infusion and during 1 hr after the end of infusion. Blood progesterone and [³H]progesterone concentrations were measured as described above.

In those animals not provided with an exteriorized carotid artery (Table 1) the concentrations measured in jugular venous blood were adjusted for extraction of progesterone by the head (mean 25 ± 3%) to give estimated arterial concentrations. A continuous exponential function was fitted to the specific activity of progesterone in arterial blood by the procedure described above.

Results from 17 experiments on pregnant ewes and 6 experiments on non-pregnant ewes are shown in Table 1. For statistical analysis the measured [³H]progesterone concentrations were adjusted to a standard infusion rate (1.0 µCi/min). They were then adjusted to a set of standard sampling times by interpolation using the individual exponential functions, retaining the deviations between measured and computed concentrations. The concentration of [³H]progesterone for the pooled

Table 1. Arterial progesterone concentration and computed [³H]progesterone time curves during and after infusion of [³H]progesterone in sheep

Sheep*	Reproductive state† (days)	Progesterone (ng/ml)	Infusion rate (µCi/min)	[³ H]progesterone‡				
				E (µCi/litre)	A	B	α (min ⁻¹)	β (min ⁻¹)
Lucy	P (7)	2.3	0.863	0.290	0.845	0.155	1.098	0.024
Merry§	P (25)	2.2	1.004	0.260	0.775	0.225	0.445	0.026
Maud§	P (27)	1.9	0.773	0.217	0.577	0.423	1.429	0.169
Lucy§	P (43)	3.7	0.628	0.236	0.695	0.305	0.299	0.014
Marigold§	P (50)	0.8	0.806	0.165	0.169	0.831	0.926	0.103
Maud§	P (62)	4.2	0.673	0.230	0.866	0.134	0.541	0.013
Frances	P (66)	4.4	0.640	0.220	0.761	0.239	0.349	0.021
Merry§	P (67)	5.1	0.845	0.214	0.653	0.347	0.455	0.038
Flossie	P (90)	6.4	0.606	0.270	0.678	0.322	0.549	0.026
Lucy	P (99)	4.9	1.121	0.370	0.695	0.305	0.325	0.022
Frances	P (103)	5.5	0.947	0.254	0.544	0.456	0.550	0.059
Madeleine	P (116)	2.5	1.174	0.337	0.728	0.272	2.987	0.043
Frances	P (122)	4.1	0.734	0.187	0.506	0.494	2.848	0.061
Flossie	P (124)	15.1	0.857	0.291	0.691	0.309	0.756	0.010
Lucy	P (134)	3.4	0.937	0.306	0.635	0.365	0.751	0.032
Frances	P (139)	3.9	0.840	0.188	0.295	0.705	7.583	0.083
Flossie	P (145)	9.6	1.344	0.390	0.541	0.459	0.698	0.035
Lucy	L (15)	1.0	1.022	0.260	0.698	0.302	0.578	0.030
Lucy	C (6)	0.2	0.787	0.285	0.834	0.166	0.354	0.009
Lucy	C (7)	3.2	0.903	0.486	0.890	0.110	0.431	0.001
Merry	C (10)	0.8	0.796	0.278	0.633	0.367	1.510	0.029
Maud	C (11)	0.8	0.786	0.241	0.673	0.327	1.830	0.048
Marigold	C (12)	3.2	0.988	0.181	0.475	0.525	1.010	0.092
Pooled (see text)			1.000	0.305	0.687	0.313	0.602	0.029

* Details of animals given by Bedford *et al.* (1972). Frances and Flossie had left adrenal transplants and the left ovary was transplanted to the neck during the first 6 weeks of pregnancy. The right ovary and right adrenal were removed before pregnancy.

† P = pregnant, Day 0 = day of mating; L = lactating, Day 0 = day of parturition; C = cyclic, Day 0 = day of ovulation.

‡ The exponential function fitted to blood [³H]progesterone is of the form:

[³H]progesterone = $E(1 - Ae^{-\alpha t} - Be^{-\beta t})$ µCi/litre during infusion, and = $E(Ae^{-\alpha t} + Be^{-\beta t})$ µCi/litre after infusion.

§ Results corrected for head extraction.

data was $0.305(1.0 - 0.687e^{-0.602t} - 0.313e^{-0.029t'})$ $\mu\text{Ci/litre}$ during infusion of [^3H]progesterone where t is time (min) from the beginning of the infusion, and $0.305(0.687e^{-0.602t'} + 0.313e^{-0.029t'})$ $\mu\text{Ci/litre}$ after the infusion where t' is time (min) after the end of the infusion.

[^3H]Progesterone concentration during infusion

The measured arterial [^3H]progesterone concentrations during the last hour of infusion were analysed in a two-factor analysis of variance of 3 replicates in each of 23 experiments. The results of this analysis are summarized in Table 2. Differences attributable to sampling times did not contribute significantly to the variance. There were significant differences between experiments, and two-factor analyses of variance for each animal showed that there were within-animal and between-animal variations. These differences could not be associated with stage of gestation or with differences between pregnant and non-pregnant animals.

Table 2. Analysis of variance of arterial [^3H]progesterone during and after infusion of [^3H]progesterone

Source	Sum of squares	d.f.	Mean square	Ratio
During infusion				
Total	0.366883	68		
Between experiments	0.315125	22	0.014324	12.86 ($P < 0.001$)
Between sample times	0.002756	2	0.001378	1.24
Residual	0.049002	44	0.001114	
After infusion				
Total	0.468485	229		
Between experiments	0.271062	22	0.012321	12.77 ($P < 0.001$)
Between sample times	0.006356	9	0.000706	0.73
Residual	0.191066	198	0.000965	

The [^3H]progesterone concentrations could be fitted to a normal distribution (Kolmogorov-Smirnov test), with a mean of 0.306 ± 0.073 (S.D.) $\mu\text{Ci/litre}$ blood.

[^3H]Progesterone concentration after infusion

The differences between measured and computed post-infusion [^3H]progesterone concentrations were considered in a two-factor analysis of variance with 10 sampling times for each of 23 experiments (Table 2). The sum of squares attributable to sampling time was not significant. There were significant differences between experiments and these could not be associated with the differences during infusion, stage of gestation or difference between pregnant and non-pregnant animals.

The differences were distributed normally (Kolmogorov-Smirnov test) with a standard deviation of 0.043 $\mu\text{Ci/litre}$ blood.

Pooled results: the metabolic clearance rate (MCR) and distribution of progesterone

Since the between-experiment differences in blood [^3H]progesterone concentration appeared to be random, the standardized values for all experiments were pooled and a common exponential function fitted (Table 3).

The computed equilibrium concentration of [^3H]progesterone for the pooled results was 0.305 ± 0.073 (S.D.) $\mu\text{Ci/litre}$ blood, and since the infusion rate was 1.0 $\mu\text{Ci/min}$ the metabolic clearance rate of progesterone was 3.277 ± 0.239 (S.D.) litre blood/min.

Clearances of progesterone by organs such as the liver and kidney are about 1.6 litre blood/min, or 50% of the MCR (Bedford *et al.*, 1974). We have assumed that progesterone in blood is part of a central pool and that tissues such as liver, kidneys and head extract progesterone from that pool.

Table 3. Observed and computed values for [³H]progesterone and [³H]20 α -dihydroprogesterone in sheep blood

Standardized sampling time* (min)	[³ H]progesterone		[³ H]20 α -dihydroprogesterone	
	Standardized conc.† (μCi/litre; mean $\pm$ S.E.M.)	Computed value‡ (μCi/litre)	Standardized conc.† (μCi/litre; mean $\pm$ S.E.M.)	Computed value§ (μCi/litre)
145	0.302 $\pm$ 0.0138	0.304	0.158 $\pm$ 0.0114	0.156
175	0.316 $\pm$ 0.0179	0.305	0.175 $\pm$ 0.0243	0.159
205	0.302 $\pm$ 0.0145	0.305	0.154 $\pm$ 0.0128	0.160
210 = infusion stopped				
0.5	0.243 $\pm$ 0.0170	0.249	0.161 $\pm$ 0.0199	0.159
1.0	0.200 $\pm$ 0.0166	0.208	0.157 $\pm$ 0.0170	0.157
2.0	0.157 $\pm$ 0.0107	0.153	0.141 $\pm$ 0.0128	0.149
3.0	0.127 $\pm$ 0.0094	0.122	0.135 $\pm$ 0.0127	0.140
4.0	0.110 $\pm$ 0.0071	0.104	0.125 $\pm$ 0.0110	0.132
5.0	0.092 $\pm$ 0.0057	0.093	0.120 $\pm$ 0.0103	0.125
15.0	0.053 $\pm$ 0.0050	0.062	0.099 $\pm$ 0.0147	0.095
30.0	0.035 $\pm$ 0.0037	0.040	0.077 $\pm$ 0.0150	0.073
45.0	0.029 $\pm$ 0.0040	0.026	0.055 $\pm$ 0.0101	0.055
60.0	0.023 $\pm$ 0.0034	0.017	0.042 $\pm$ 0.0097	0.040

* Concentrations adjusted by exponential interpolation (see text).

† Standardized to infusion rate of 1.0 μCi/min.

‡ Fitted exponential function $0.305(0.687e^{-0.602t} + 0.313e^{-0.029t})$ after infusion.

§ Numerical solution of differential equations.

The second pool may be regarded as an outer pool and the remaining 50% of the MCR must occur there. This constraint enables all the parameters of the two-pool model to be determined; these are shown in Table 4.

Table 4. Parameters of a two-pool model of progesterone distribution in sheep derived from the exponential function fitted to the pooled results (means $\pm$ S.E.M.) from 23 experiments

* $k_{01} = 0.211 \pm 0.020 \text{ min}^{-1}$;	For [P] = 1.0 μg/litre,
$k_{21} = 0.378 \pm 0.086 \text{ min}^{-1}$;	$f_{01} = 1.639 \text{ μg/min}$
$k_{12} = 0.0185 \pm 0.0067 \text{ min}^{-1}$;	$f_{21} = 2.937 \text{ μg/min}$
$k_{02} = 0.0233 \pm 0.0049 \text{ min}^{-1}$;	$f_{12} = 1.298 \text{ μg/min}$
	$f_{02} = 1.639 \text{ μg/min}$
* $V_1 = 7.76 \pm 0.808 \text{ l blood equivalent}$;	$V_1[P] = 7.76 \text{ μg}$
$V_2 = 70.2 \pm 10.7 \text{ l blood equivalent}$;	$V_2[P] = 70.2 \text{ μg}$

For an infusion rate of 1.0 μCi/min, [³H]progesterone = $0.305(1.0 - 0.687e^{-0.602t} - 0.313e^{-0.029t})$ during infusion, and $0.305(0.687e^{-0.602t} + 0.313e^{-0.029t})$ after infusion. The standard errors of the parameters were derived from the residual variance and the variance-covariance matrix generated in curve fitting.

* k_{ij} is the rate constant for flux from pool j to pool i; the pool sizes (μg progesterone) are represented by their equivalent volumes of arterial blood (V_i) multiplied by the blood concentration of progesterone [P]. f_{ij} is the flux of progesterone (μg/min) from pool j to pool i.

20 α -Dihydroprogesterone

Bedford *et al.* (1973) observed a close correlation between the equilibrium blood concentrations of [³H]progesterone and [³H]20 α -diHP and noted that the slope of the regression line was similar to that observed by Short & Moore (1959) for the endogenous concentrations of these steroids in blood. The correlation coefficients of labelled progesterone and 20 α -diHP, and of endogenous

progesterone and 20 α -diHP concentrations were not significantly different (Fisher's z test, $P > 0.25$). Accordingly, in deriving the model described in this paper we have assumed that progesterone is the predominant source of circulating 20 α -diHP, and that at equilibrium during infusion of labelled progesterone the specific radioactivities of the two steroids are the same.

The arterial blood concentrations of [^3H]20 α -diHP were adjusted to a standard infusion rate (1.0 $\mu\text{Ci}/\text{min}$) and to standard sampling times; the results are shown in Table 3.

The formation and distribution of 20 α -diHP

In fitting the numerical solutions of Equations 8–11 to the observed 20 α -diHP concentrations, some of the parameters, V_1 , V_2 , k_{21} and k_{12} can be obtained from Table 4. For this four-pool model, $(k_{01} + k_{41})$ and $(k_{02} + k_{32})$ were constrained to equal k_{01} and k_{02} respectively of Table 4. From the measured arterial equilibrium concentrations of [^3H]progesterone and [^3H]20 α -diHP concentrations, $[\text{D}] = 0.529[\text{P}]$.

In trials of the pairs of rate constants, k_{14} and k_{41} and k_{32} and k_{23} , it appeared likely that there was little re-conversion of 20 α -diHP to progesterone. This was particularly evident in the very poor fit of the computed function and the observed concentrations of labelled 20 α -diHP for values of k_{41} and k_{23} except when these were very small. The solution was not particularly sensitive to changes in the exchange of 20 α -diHP between pools 3 and 4. It was the four rate constants k_{0i} which had the greatest effect on the fit to the observed concentrations of labelled 20 α -diHP.

The final choice of parameters was influenced by the following considerations: k_{01} was chosen to give a clearance of progesterone equal to 30% MCR, which is only slightly greater than the measured splanchnic clearance of progesterone (Bedford *et al.*, 1974); k_{04} was chosen to give a similar clearance for 20 α -diHP; k_{02} was chosen to be $(1.0 - 0.529)$ of the clearance of progesterone in the outer pool and k_{03} was adjusted to give the required total MCR.

The final parameters and model are shown in Table 5 and the agreement between measured and computed [^3H]progesterone and [^3H]20 α -diHP concentrations is shown in Text-fig. 1. The sum of squares of deviations between measured and computed values was 0.000448 (S.D. = 0.006) for progesterone and 0.000478 (S.D. = 0.006) for 20 α -diHP.

The parameters shown in Table 5 are not unique, but they do provide a near-minimum sum of squares of deviations for 20 α -diHP.

Table 5. Parameters of a four-pool model of progesterone [P] and 20 α -dihydroprogesterone (20 α -diHP) [D] distribution and metabolism in sheep, obtained using Equations 8–11 (see text)

$k_{01} = 0.1288 \text{ min}^{-1}$;	$f_{01} = 1.000 \text{ } \mu\text{g}/\text{min}$	$k_{21} = 0.3783 \text{ min}^{-1}$;	$f_{21} = 2.937 \text{ } \mu\text{g}/\text{min}$
$k_{02} = 0.0110 \text{ min}^{-1}$;	$f_{02} = 0.771 \text{ } \mu\text{g}/\text{min}$	$k_{23} = \text{small}^*$	
$k_{03} = 0.0263 \text{ min}^{-1}$;	$f_{03} = 0.977 \text{ } \mu\text{g}/\text{min}$	$k_{32} = 0.0124 \text{ min}^{-1}$;	$f_{32} = 0.868 \text{ } \mu\text{g}/\text{min}$
$k_{04} = 0.1288 \text{ min}^{-1}$;	$f_{04} = 0.529 \text{ } \mu\text{g}/\text{min}$	$k_{34} = 0.1938 \text{ min}^{-1}$;	$f_{34} = 0.796 \text{ } \mu\text{g}/\text{min}$
$k_{12} = 0.0185 \text{ min}^{-1}$;	$f_{12} = 1.298 \text{ } \mu\text{g}/\text{min}$	$k_{41} = 0.0823 \text{ min}^{-1}$;	$f_{41} = 0.639 \text{ } \mu\text{g}/\text{min}$
$k_{14} = \text{small}^*$		$k_{43} = 0.0185 \text{ min}^{-1}$;	$f_{43} = 0.687 \text{ } \mu\text{g}/\text{min}$
For $[\text{P}] = 1.0 \text{ } \mu\text{g}/\text{litre}$, $[\text{D}] = 0.53 \text{ } \mu\text{g}/\text{litre}$ and $s = 3.277[\text{P}] \text{ } \mu\text{g}/\text{min}$,			
$V_1[\text{P}] = 7.76 \text{ } \mu\text{g}$		$V_2[\text{P}] = 70.2 \text{ } \mu\text{g}$	
$V_1[\text{D}] = 4.11 \text{ } \mu\text{g}$		$V_2[\text{D}] = 37.2 \text{ } \mu\text{g}$	

Some of these parameters were taken from Table 4, and the remainder were obtained by adjustments which minimized the sum of squares of differences between measured and computed [^3H]20 α -diHP concentrations. All values are given in terms of progesterone. All fluxes of 20 α -diHP are expressed as equivalents of progesterone, and should be divided by 0.529 to give flux as [D].

* Net exchanges were $<0.1 \text{ } \mu\text{g}/\text{min}$.

Discussion

The blood concentrations of [^3H]progesterone and [^3H]20 α -dihydroprogesterone during and after the infusion of [^3H]progesterone accord with the hypothesis that progesterone is distributed within

Text-fig. 1. Measured blood concentrations of [³H]progesterone (○) and [³H]20α-dihydroprogesterone (●), and the computed line for the blood concentration of [³H]progesterone (---) and [³H]20α-dihydroprogesterone (—) during and after the infusion of [³H]progesterone. The computed values were obtained from Equations 8–11 (see text) and the parameters given in Table 5.

the body between two compartments. The amount of progesterone associated with a central pool, 7.8[P] μg, indicates a compartment greater than that of the vascular system and which in this study has been taken to include tissues such as liver, kidneys and head where the hormone is rapidly catabolized. In previous studies it was estimated that the clearance of progesterone within this pool represents about 50% of MCR (Bedford *et al.*, 1974), so that the remaining 50% of catabolism occurs in a second, or outer pool whose dimension is apparently large, 70[P] μg. The difference between the computed values for these two pools contrasts with the figures derived by Little *et al.* (1966) in human subjects in which values of about 12[P] μg and 15[P] to 27[P] μg were obtained for the central and outer pools, respectively. The mean body weight of sheep used in our study, however, was 41 kg compared with a value of about 80 kg in the subjects investigated by Little *et al.* (1966). The dimension of the central pool corrected for body weight was therefore similar in the two species. Little *et al.* (1966) concluded that the rapid disappearance of progesterone from blood is caused by its rapid rate of metabolism. The large size of the outer pool in sheep implies that the extravascular concentration of progesterone is much greater than that in blood, and this may reflect the high lipid solubility of progesterone (99.3 μmol/μmol phosphatidylcholine: Heap, Symons & Watkins, 1970) and the presence of intracellular receptors or binding sites. The results indicate a clear species difference in the distribution of progesterone in an outer pool.

Another feature of progesterone metabolism in sheep which differs from that in man concerns the relatively high proportion of 20α-diHP in circulation. The percentage conversion ratio of 20α-diHP:progesterone is at least 5 times greater in sheep (Bedford *et al.*, 1972) than in women

(Lin, Billiar & Little, 1972). In sheep progesterone appears to be the major source of 20 α -diHP, but does not seem to be an obligatory catabolite.

It was not practicable to optimize the parameters of a four-pool model mathematically, but those obtained gave equally low residual deviations for both labelled steroids and are consistent with other observations made on progesterone clearance. The clearance of progesterone from the central pool which does not produce 20 α -diHP, ($k_{01} V_1[P]a = 1.0$ litre blood/min = 33% MCR), is of the same order as splanchnic clearance of progesterone (1.155 litre/min: Bedford *et al.*, 1974). The clearance of 20 α -diHP from the central pool, ($k_{04} V_1[D]a_4 = 1.0$ litres blood/min), is of the same order as the observed splanchnic clearance of this steroid (1.138 litres/min). The computed rate of conversion of progesterone to 20 α -diHP in the central pool is greater than has been observed experimentally. However, this value was obtained from pooled results which came predominantly from pregnant ewes. The conversion to 20 α -diHP by the gravid uterus and by the kidney has not yet been studied in depth, and might be sufficient to account for the computed rate. Greater precision should also be achieved when measurements of blood specific activity of 20 α -diHP have been made during and after the infusion of [3 H]20 α -diHP.

Criticism has been made of earlier work that the estimated MCR of progesterone was of the same order as cardiac output, and therefore physiologically dubious. In previous experiments it was shown that progesterone metabolism by maternal erythrocytes was negligible (Bedford *et al.*, 1972) and unlikely to represent a significant source of error in the estimation of MCR. The current results also provide confirmation that steady state conditions were achieved since the mean MCR of progesterone computed from blood concentrations during and after the infusion (3.277 litres/min) was very similar to that calculated for the same experiments from blood concentrations during the infusion alone (3.397 litres/min; Bedford *et al.*, 1972). The total efflux of progesterone from the central pool, ($k_{01} + k_{21} + k_{41}) V_1[P]$, is 4.58 litres blood/min and this comprises 1.53 litres/min at very high extraction efficiency with the remainder at about 56% extraction. Although this total efflux is not much smaller than cardiac output its validity could be checked in further experiments. Our model predicts that most major blood flows in the body should show an average extraction of 56% progesterone (excluding the splanchnic region). The model also requires that there should be a significant source of 20 α -diHP in the central pool in pregnant animals, and the uptake of progesterone by the gravid uterus should be investigated.

Further validation of the assumptions on which the model is based will be tested by the infusion of labelled 20 α -diHP. The model predicts that the blood specific activity of 20 α -diHP should be described by a double exponential, the outer pool of 20 α -diHP should be about 10 times greater than the central pool, and there should be little if any formation of labelled progesterone.

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