Disappearance and unexpected reappearance of progesterone in the circulation of the monkey: novel hormone kinetics

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1. Intravenous injection of [^{3}H]progesterone in non-pregnant monkeys resulted in total disappearance of the labelled hormone from the circulation within 3 h. However, 0·5–1·75 h after disappearance the hormone reappeared, reaching 20% (median, 5%) of the initial maximal concentration.

2. Reappearance of labelled hormone was accompanied by similar fluctuations in the levels of labelled metabolites, [^{3}H]20α-dihydroprogesterone and [^{3}H]17α-hydroxyprogesterone, which reached 61% (median, 14%) and 120% (median, 13%), respectively, of the initial maximal concentrations.

3. Chromatography was used to separate labelled progesterone and its metabolites. Efficiency of the procedure was determined separately in each sample and for each steroid. All data were corrected for percentage recovery.

4. Analytical equations were devised, based on the theory of compartmental systems with continuously distributed time lags, to describe the unexpected kinetics of progesterone levels. The coefficients of determination ranged from 86 to 99% (median, 96%) which indicates that the equations enabled reliable prediction of hormone levels in blood within the time range studied.

5. The unexpected reappearance of labelled progesterone cannot be explained by hormone secretion but only by a delayed release from tissue stores, since progesterone does not undergo enterohepatic recirculation. Thus, a previously undescribed mechanism affecting circulating progesterone levels, and perhaps those of other hormones, exists.

Progesterone is the principal steroid hormone secreted by the ovary during the luteal phase of the menstrual cycle in primates (Wentz, 1988; MacDonald, Dombroski & Casey, 1991). It plays a pivotal role in reproduction, being necessary for the implantation of the embryo in the uterus and the maintenance of subsequent pregnancy (Wentz, 1988). In addition, it exerts multiple metabolic and regulatory actions on the body which affect such diverse physiological processes as hormone secretion by the pituitary gland, respiration and carbohydrate metabolism, among many others (MacDonald *et al.* 1991; Goldfien & Monroe, 1994).

It is generally accepted that the appearance of any hormone in the circulation is associated with secretion from the endocrine gland. In addition, some steroid hormones undergo enterohepatic circulation, which also results in the appearance of hormone in the blood (Taylor, 1971; Adlercreutz & Martin, 1980). Hormones may also be formed in tissues other than endocrine glands, such as adipose or muscular tissue (O'Malley & Stroitt, 1991). An example is that of oestrogen (Taylor, 1971; Adlercreutz & Martin, 1980; O'Malley & Stroitt, 1991). Progesterone, however, does not undergo enterohepatic circulation (Taylor, 1971; Adlercreutz & Martin, 1980) nor is it released from organs other than the ovary in any significant amount, at least in the non-pregnant female (MacDonald *et al.* 1991). The kinetics of [^{3}H]progesterone after a single input into the circulation of the monkey reported here is, therefore, unexpected.

Hormone levels in blood can be characterized mathematically by compartmental analysis (Godfrey, 1983). According to such analysis, following appearance in the circulation, hormone is distributed in the body within one or more homogenous anatomical spaces referred to as compartments.

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If the rates of hormonal flow to and from a compartment are proportional to the hormone concentration in a donor compartment, and if the rate coefficients are constant over time, then the equations arising from compartmental models may describe a constant exponential decline of hormone levels, their non-periodic fluctuations or, for systems of at least three compartments, sinusoidal oscillations (Gurpide & Mann, 1970; Thron, 1972; Smith & Moller, 1976; Godfrey, 1983). Increases in the circulating hormone levels may, therefore, result from: (i) release from a gland; (ii) extra-glandular formation; (iii) enterohepatic circulation; or theoretically (iv) redistribution either in the form of periodic oscillations or other fluctuations due to the intrinsic characteristics of compartmental systems. To our knowledge, no experimental evidence for (iv) has ever been documented for any hormone, including progesterone (Gurpide & Mann, 1970; Thron, 1972; Smith & Moller, 1976). Thus, the novelty of our finding is the reappearance in the circulation of a steroid hormone, progesterone, which was not due to circumstances (i)–(iii). Our unexpected finding was made during studies on the effects of stress hormones on reproductive function in primates (Kowalski & Chatterton, 1993; Kowalski, Chatterton, Kazer & Wentz, 1993).

METHODS

Experimental procedures

Animals. Seven adult female cynomolgus monkeys (*Macaca fascicularis*) from Charles River Laboratories (Wilmington, MA, USA), weighing 2·5–3·6 kg, were used in a study on the effects of a stress hormone, cortisol, on reproduction. They received food and water *ad libitum* and were caged individually in a room with controlled temperature (26–27 °C) and lighting (lights on from 05.00 to 19.00 h). Experimental procedures were approved by the Animal Care and Use Committee of Northwestern University. They involved subcutaneous implantation of osmotic pumps, for approximately 2 months, which released hydrocortisone phosphate at 15 mg day^{-1} or saline, as described elsewhere (Chatterton, Kazer & Rebar, 1991; Kowalski & Chatterton, 1993; Kowalski *et al.* 1993). The fitting of the pumps, as well as the collection of blood samples for hormone radio-immunoassays to monitor menstrual cycles, were done under sedation with intramuscular ketamine (10 mg kg^{-1}) and xylazine (0·5 mg kg^{-1}). In the mid-luteal phase of the menstrual cycle (i.e. on the 8th ± 1 day after the serum oestradiol peak), [1α,2α(*n*)-^{3}H]progesterone (specific activity 47·8 Ci $mmol^{-1}$; Amersham, Arlington Heights, IL, USA) was rapidly injected into the cubital vein from a 1 ml glass tuberculin syringe, at a dose of 50 μCi in 0·5 ml of aqueous 65% (v/v) propylene glycol. Injection of labelled hormone was accomplished by direct needle puncture into the vein and was followed by a 6 h blood sampling period from a catheter inserted into the contralateral vein. Blood samples were taken every 5 min for the first 30 min (1 ml) and every 15 min thereafter (2·5 ml). Fluid balance was maintained equivolumetrically (1 ml or 2·5 ml, as appropriate) with heparinized saline (10 U ml^{-1}) to keep the catheter patent. Experiments were done under 1% isoflurane anaesthesia with carrier oxygen (1 l) and nitrous oxide (1 l), after premedication with intramuscular ketamine (10 mg kg^{-1}) and xylazine (0·5 mg kg^{-1}). The duration of anaesthesia did not exceed 7 h. No postoperative care was required. At the end of each trial animals received an intramuscular injection of iron (equivalent to 50 mg of elemental iron). Isoflurane was chosen for the study because it preserves the cardiac output, the renal blood flow and the liver blood flow, only metabolizes minimally (less than 1%) and does not alter hepatic function (Jones, 1994). Fifteen trials were performed: once at the end of hydrocortisone treatment and once during saline infusion in each monkey and, additionally, one more time during saline infusion in one animal. Individual trials in the same animal were separated by 1–3 months. The study was always begun between 09.00 and 10.00 h.

Separation of serum [^{3}H]steroids. Serum [^{3}H]progesterone and its metabolites, [^{3}H]20α-dihydroprogesterone ([^{3}H]20α-DP) and [^{3}H]17α-hydroxyprogesterone ([^{3}H]17α-HP), were extracted and separated by thin-layer chromatography. Recoveries were determined separately for each steroid in each sample. Glass tubes for extraction with carrier progesterone, 20α-DP and 17α-HP (Sigma) were prepared by adding 50 μl of the 1 mg ml^{-1} methanol solutions of each carrier steroid, followed by evaporation of methanol under nitrogen. Aliquots of serum (200 μl) were extracted twice with 1 ml of ethyl ether, and the extract was transferred to polypropylene tubes. The ethyl ether from the combined extracts was evaporated under nitrogen and the residue was transferred twice, in 100 μl of ethyl acetate, to the plates containing a short-wave indicator, fluorescent at 254 nm (Brinkmann, Westbury, NY, USA). The plates were developed twice in a system of benzene : acetone (10 : 3, v/v) which was prepared immediately before use. Identification of carrier steroids on the plate was made under a UV lamp. The separation of all three steroids was complete under the system of organic solvents employed in this study. Purity of the progesterone separation had been confirmed previously by successive co-crystallization to a constant specific activity with the authentic steroids (Braasch, Frederiksen & Chatterton, 1988). Areas corresponding to progesterone, 20α-DP and 17α-HP were scraped and transferred to separate glass tubes for extraction with 2·5 ml of high-pressure liquid chromatography grade methanol. Progesterone, 20α-DP and 17α-HP were measured in the methanolic solutions by absorbances at 240 nm in a spectrophotometer to determine recovery of the added carriers. The solutions were quantitatively transferred to scintillation vials and the methanol was evaporated to dryness. The vials and the background blanks were counted in a liquid scintillation counter with a 40% counting efficiency. All samples from a given trial were always analysed in the same batch. Median recoveries of the added carrier steroids for all three procedural steps were 44% for progesterone, 45% for 20α-DP and 46% for 17α-HP, which corresponded to mean recoveries for a single procedural step of 76, 77 and 77%, respectively.

Equations

Simple compartmental systems. Experimental data for [^{3}H]progesterone were fitted with equations arising from linear, time-invariant compartmental models (Gibaldi & Perrier, 1982; Godfrey, 1983) by the least-squares method (the MINSQ program for non-linear parameter estimation from MicroMath; Salt Lake City, UT, USA). Two cases were distinguished depending on whether a given trial injection of the labelled hormone occurred as a perfect bolus (within a very short time interval, equal to zero) or not (over a short time interval, $r > 0$, not exceeding a few seconds, and at a constant infusion rate). An important difference between the perfect bolus and the ultra-short infusion at a constant rate is that in the former case, hormone concentration in the circulation is maximal at time zero, while in the latter case, the concentration is zero at time zero. Both cases (perfect bolus and ultra-short infusion)

Table 1. Kinetics of [^{3}H]progesterone and its metabolites

		[^{3}H]Progesterone			[^{3}H]20α-DP			[^{3}H]17α-HP		
		Median (min)	Range (min)	c.v. (%)	Median (min)	Range (min)	c.v. (%)	Median (min)	Range (min)	c.v. (%)
Disappearance										
Duration	I	120	90–180	19	165	90–225	17	90	60–180	33
Reappearance										
Beginning	II	195	150–255	7	225	180–315	7	270	240–345	8
Delay	III	75	30–105	39	45	30–165	69	195	75–225	17
Termination	IV	240	225–300	8	300	270–360	10	300	255–360	11
Duration	V	45	30–135	50	75	30–165	50	30	15–120	93

Median and range values are in minutes; coefficients of variation (c.v.) are about the median.Values in lines I, II and IV were calculated in relation to the time of [^{3}H]progesterone administration. I was determined as the last time point in which the levels were above zero or the nadir; II was determined as the first time point in which the levels were above zero or the nadir; III was calculated as difference between II and I, separately for each trial; IV was determined as the last time point in which the levels were above zero; V was calculated as the difference between IV and II, separately for each trial. For the [^{3}H]17α-HP, rows II–V represent 9 of 15 trials in which it reappeared.

are a good representation of endogenous hormone secretion, which occurs episodically in a burst-like manner (Veldhuis, 1991; Pincus & Keefe, 1992). To avoid redundancy of equation parameters, the data were modelled with a maximum of four compartments. The value of r was always assumed to be between 0·001 and 0·100 min. However, none of the equations (Gibaldi & Perrier, 1982; Godfrey, 1983), including that for sinusoidal oscillations, fit the data adequately. (Coefficients of determination were low or the reappearance of [^{3}H]progesterone could not be modelled by the equation. In addition, these equations frequently predicted negative values for the concentration within the sampling interval of 5–360 min and/or before or beyond that interval. The initial phase of [^{3}H]progesterone distribution and elimination was, however, modelled well by a simple two-compartmental system.)

Compartmental systems with continuously distributed time lags. Mathematical characterization of the [^{3}H]progesterone levels in blood was achieved using a convolution integral to account for the time lag between the labelled hormone disappearance and its reappearance (MacDonald, 1978; Godfrey, 1983). To avoid redundant equation parameters, one- and two-compartmental systems were used for the cases of perfect bolus ($r = 0$) and ultra-short infusion ($r > 0$). Equations and their parameters are defined in the Appendix. Fitting the data was performed as described for simple compartmental systems.

RESULTS

Experimental procedures

Kinetics of [^{3}H]progesterone and its metabolites. Figure 1 exemplifies the unexpected kinetics of serum [^{3}H]progesterone, [^{3}H]20α-DP and [^{3}H]17α-HP. The two treatment regimes (hydrocortisone *versus* saline) did not differ with regard to the redistribution parameters of [^{3}H]progesterone and its metabolites, therefore data were combined. (Hydrocortisone, however, increases the metabolic clearance rate and the volume of distribution of progesterone without affecting its elimination constant, Kowalski *et al.* 1993.) Temporal parameters for the disappearance and reappearance of [^{3}H]progesterone and its metabolites in the circulation (median and range) are shown in Table 1. Total disappearance from the circulation occurred in thirteen trials for the [^{3}H]progesterone, in eleven trials for the [^{3}H]20α-DP, and in all the trials with [^{3}H]17α-HP. The [^{3}H]20α-DP reappeared 30 min (range, 15–60 min) after the reappearance of [^{3}H]progesterone. The [^{3}H]17α-HP did not reappear in six trials and in the remaining nine trials, it reappeared 90 min (range, 60–150 min) after the reappearance of [^{3}H]progesterone. During the reappearance, [^{3}H]progesterone reached a median of 5% (range, 0·3–20%) of the initial maximal concentration; those values for [^{3}H]20α-DP and [^{3}H]17α-HP were 14% (range, 2–61%) and 13% (range, 0–120%), respectively. Relative amplitudes (in percentages) of [^{3}H]progesterone during the reappearance were positively correlated with relative amplitudes of the metabolites. The Spearman rank correlation coefficient (R) was 0·73 ($n = 15$; $P < 0·005$) between the amplitudes of [^{3}H]progesterone and [^{3}H]20α-DP, and 0·58 ($n = 15$; $P < 0·025$) between the amplitudes of [^{3}H]progesterone and [^{3}H]17α-HP. There was no statistically significant correlation between relative amplitudes of the metabolites ($R = 0·48$; $n = 15$; $P > 0·05$).

Equations

Compartmental systems with continuously distributed time lags. Figure 2 represents compartmental systems that were used to describe the serum levels of [^{3}H]progesterone. Equations for the ultra-short infusion (eqns (A7) and (A8)) generally represented data better than those for the perfect bolus (eqns (A5) and (A6)). The best representation of the data for [^{3}H]progesterone with regard to coefficients of determination and physiological plausibility (the predicted values for the concentration must be non-negative) was obtained with eqn (A7) (representing one compartment) for thirteen trials in six monkeys and with eqn (A8) (representing two compartments) for two trials in one monkey. In those cases, coefficients of determination ranged

from 86 to 99% (median, 96%) and statistical analysis of residuals gave the most satisfactory correlation (serial correlation coefficients were not significant at $P < 0{\cdot}01$). Means and 95% confidence intervals for equation parameters in those cases ($n = 15$) were 0·076 min (0·053–0·099) for r, 192 min (181–203) for s (time after which reappearance of [^{3}H]progesterone began in relation to r), and 0·066 min^{-1} (0·037–0·095) for m (elimination constant of [^{3}H]progesterone; in the case of eqn (A8), terminal elimination constant). The least variable parameter among all fifteen trials was s, for which the coefficient of variation (c.v.) about the mean was 10%. The coefficients of variation for parameters m and r were 79 and 55%, respectively. When the within-subject variability between repeated trials in the same monkey was estimated with regard to the physiological parameters s and m, the c.v. for s remained the same while that for the elimination constant was decreased to 48%.

DISCUSSION

Since the injected hormone was labelled, its reappearance was not due to secretion from the ovary. The reappearance of [^{3}H]progesterone in the circulation must, therefore, have resulted from a prior accumulation in a tissue reservoir (or reservoirs) such as the adipose and muscular tissues, with

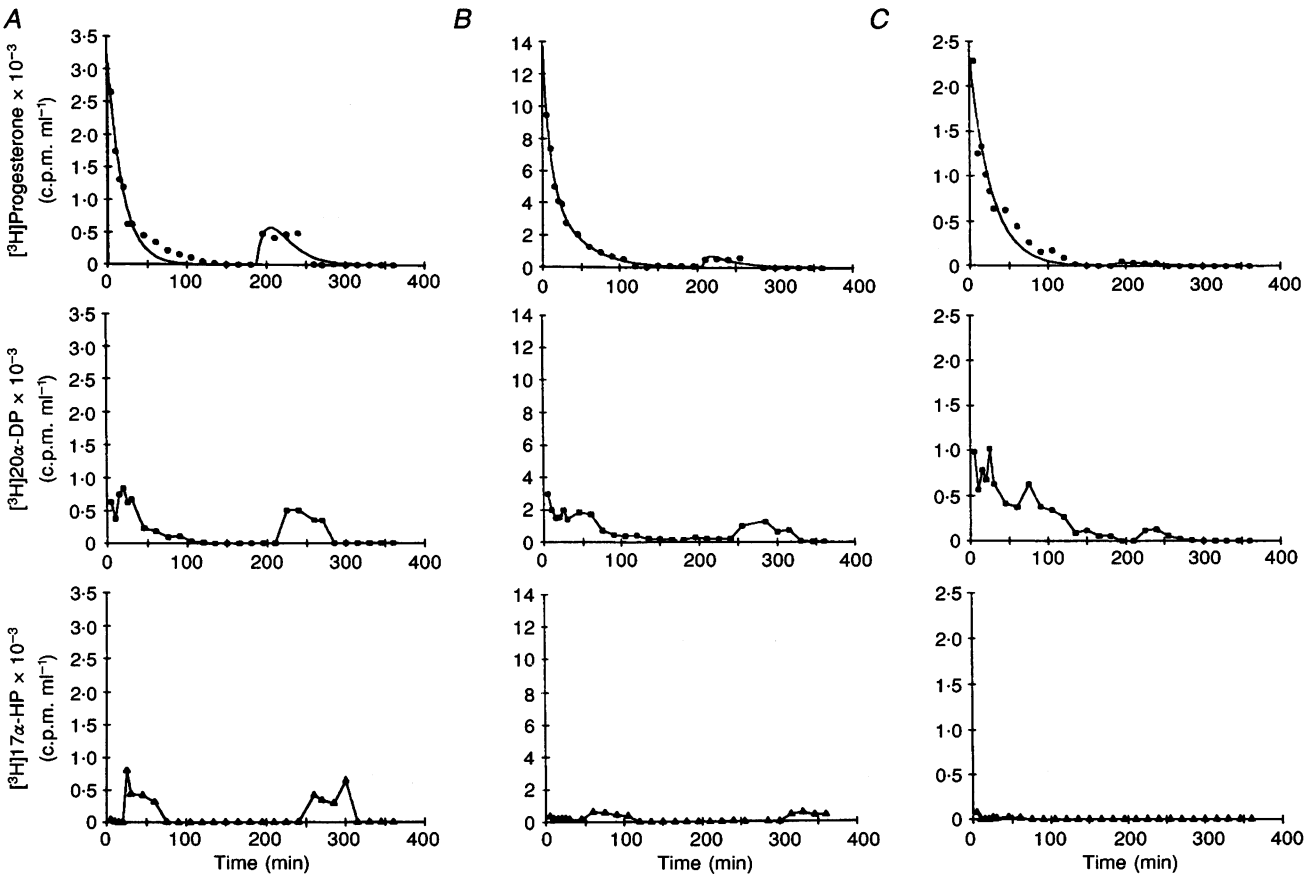

Figure 1. The serum levels of [^{3}H]progesterone, [^{3}H]20α-DP and [^{3}H]17α-HP in three trials

Serum levels with the high (*A*), middle (*B*) and low (*C*) relative amplitudes of the redistribution phase in c.p.m. are shown. The data for [^{3}H]progesterone (*A*) are described by eqn (A7) with the parameters: $A = 59{\cdot}4 \times 10^6$ c.p.m. ml^{-1}, $G = 0{\cdot}026$ c.p.m. ml^{-1}, $m = 0{\cdot}055$ min^{-1}, $r = 0{\cdot}001$ min and $s = 187$ min (coefficient of determination = 97%). See Appendix for definitions. The equation is represented by the continuous line in the figure. The data for [^{3}H]progesterone (*B*) are described by eqn (A8) with the parameters: $A = 2{\cdot}3 \times 10^6$ c.p.m. ml^{-1}, $B = 0{\cdot}7 \times 10^6$ c.p.m. ml^{-1}, $G = 0{\cdot}001$ c.p.m. ml^{-1}, $H = 0{\cdot}011$ c.p.m. ml^{-1}, $m = 0{\cdot}025$ min^{-1}, $k = 0{\cdot}118$ min^{-1}, $r = 0{\cdot}100$ min and $s = 206$ min (coefficient of determination = 99%). The equation is represented by the continuous line in the figure. The data for [^{3}H]progesterone (*C*) are described by eqn (A7) with the parameters: $A = 0{\cdot}6 \times 10^6$ c.p.m. ml^{-1}, $G = 0{\cdot}002$ c.p.m. ml^{-1}, $m = 0{\cdot}037$ min^{-1}, $r = 0{\cdot}100$ min and $s = 179$ min (coefficient of determination = 95%). The equation is represented by the continuous line in the figure.

subsequent release into the bloodstream. Since progesterone is the most hydrophobic of all hormones (Kincl, 1971) it is the one that would be most likely to accumulate in the adipose tissue. Indeed, synthetic analogues of progesterone have been shown to accumulate in the subcutaneous fat (Kuhl, 1990).

Hormones are secreted episodically in a pulsatile manner (Veldhuis, 1991; Pincus & Keefe, 1992). The results of this study indicate, however, that pulses of progesterone in the circulation (Healy, Schenken, Lynch, Williams & Hodgen, 1984; Rossmanith, Laughlin, Mortola, Johnson, Veldhuis & Yen, 1990) may result not only from episodes of secretion but also from a delayed release from tissue stores. Therefore, our finding could explain why, in fact, so many progesterone pulses in the circulating blood are not associated with corresponding pulses of luteinizing hormone (LH; Healy *et al.* 1984; Rossmanith *et al.* 1990), which is secreted by the pituitary gland and regulates luteal function and progesterone secretion (Wentz, 1988).

The study was performed in the mid-luteal phase of the menstrual cycle, at the time when endogenous progesterone secretion is maximal (Wentz, 1988; MacDonald *et al.* 1991). The administered labelled hormone was therefore mixed in the circulation with the endogenous one. It is obvious that only infusion of a labelled hormone can detect redistribution phenomena. Analysis of an endogenous hormone profile or

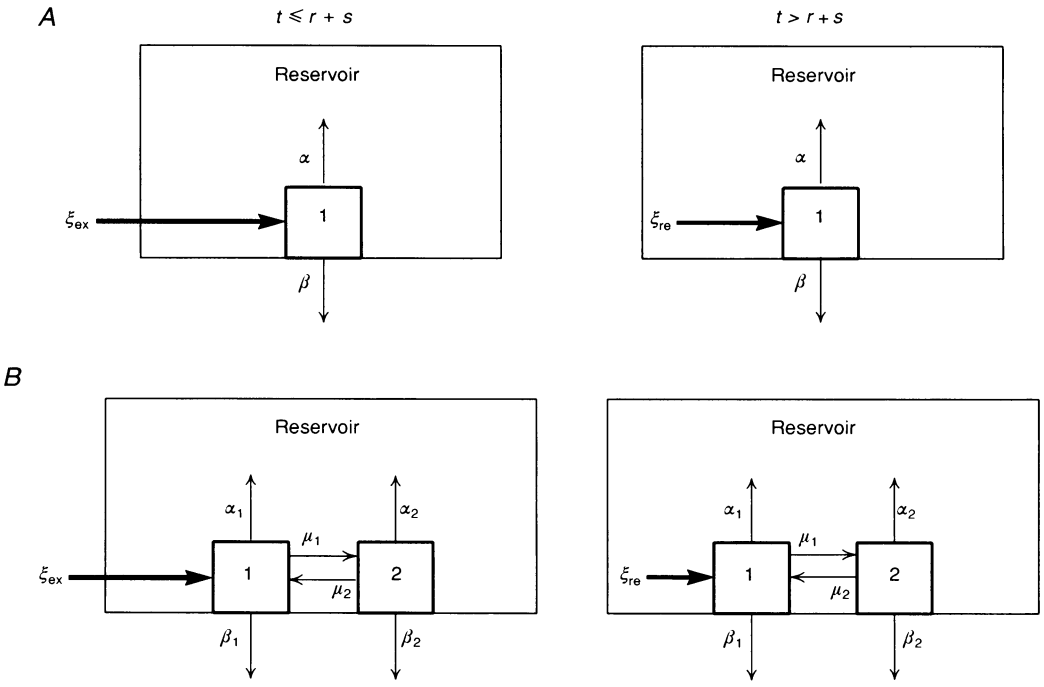

Figure 2. Graphical representation of compartmental systems that were used to describe concentration of [^{3}H]progesterone in the circulation

A, one-compartmental system. The flow rate from the compartment is proportional to the hormone concentration. Constants α and β (α, $\beta > 0$) denote the flow of hormone to a tissue reservoir and the irreversible loss from the body through metabolism or another elimination route, respectively. This system is represented by eqns (A5) and (A7), in which $m = \alpha + \beta$. *B*, two-compartmental system. The flow rate between the compartments is proportional to the hormone concentration in the donor compartment and is represented by constants μ_1 and μ_2 (μ_1, $\mu_2 \geqslant 0$; $\mu_1\mu_2 \neq 0$). Constants α_i and β_i ($i = 1$ or 2; α_i, $\beta_i \geqslant 0$; $\alpha_1\alpha_2 \neq 0$; $\beta_1\beta_2 \neq 0$) denote the flow of hormone to a tissue reservoir and the irreversible loss from the body through metabolism or another elimination route, respectively. Necessary and sufficient conditions for the system to have a single real characteristic function are (Gibaldi & Perrier, 1982): (i) $\mu_2 = 0$ (unidirectional flow between the compartments), and (ii) $\alpha_1 + \beta_1 + \mu_1 = \alpha_2 + \beta_2$. Otherwise, the system has two real distinct characteristic functions. For a single real characteristic function, the system is represented by eqns (A5) and (A7), in which $m = \alpha_1 + \beta_1 + \mu_1 = \alpha_2 + \beta_2$. For two real distinct characteristic functions, the system is represented by eqns (A6) and (A8), in which $m,k = \{(Q_1 + Q_2) \pm [(Q_1 - Q_2)^2 + 4\,\mu_1\mu_2]^{0.5}\}/2$, where $Q_1 = \alpha_1 + \beta_1 + \mu_1$ and $Q_2 = \alpha_2 + \beta_2 + \mu_2$. '1' represents the central compartment (the circulation) and '2' the peripheral compartment (the extravascular space and tissues). ξ_{ex}, exogenous input of [^{3}H]progesterone into the circulation; ξ_{re}, endogenous input of [^{3}H]progesterone into the circulation after time $r + s$ from the tissue reservoir (redistribution).

an infusion of unlabelled hormone cannot provide such evidence since neither can differentiate between the episode of secretion from a gland and the release from a tissue store.

The redistribution phenomenon of progesterone could not be fitted mathematically by equations resulting from simple compartmental analysis, in particular by equations that describe sinusoidal oscillations (Gurpide & Mann, 1970; Thron, 1972; Smith & Moller, 1976; Godfrey, 1983), but required the use of a convolution integral. The convolution integral was employed in this study on the basis of a general theory of dynamic systems (Godfrey, 1983). Our finding that two equations (rather than a single one) had to be used to best describe the data is not surprising, since the number of compartments for the same substance may differ among individuals and may even vary within the same individual on different occasions (Gibaldi, 1991). An extended and more frequent collection of blood samples may overcome such uncertainty (Godfrey, 1985; Landaw, 1985; Gibaldi, 1991). Since the equations fit the data well from the statistical point of view, they can be reliably used to predict progesterone levels in blood after burst-like input within the time range studied. They do not, however, provide an explanation for the redistribution phenomenon of progesterone and, thus, they do not constitute a physical (or chemical) model. Creation of such model for this phenomenon is under investigation.

The constancy of temporal parameters for the reappearance of [^{3}H]progesterone and its metabolites in blood, as evidenced by the low c.v.s in Table 1 (rows II and IV) and by the low c.v. for the predicted value of s (based on eqns (A7) and (A8)), was striking. Such a small variability suggests the importance of the redistribution phenomenon. Whether other hormones, and specifically steroid hormones, undergo a disappearance–reappearance cycle (or cycles) is an open question. An answer to this question is important because steroid hormones are involved in a complex network of negative and positive feedbacks on the secretion of their regulatory hormones by the pituitary gland. For example, progesterone and oestradiol inhibit or stimulate (depending on the phase of the menstrual cycle) LH secretion in females (Plant, 1986), testosterone inhibits LH secretion in males (Plant, 1986), and cortisol inhibits adrenocorticotrophin secretion (Keller-Wood & Dallman, 1984). It is possible that the redistribution phenomenon of steroid hormones may impact upon such regulatory feedback loops.

Progesterone is necessary for successful pregnancy (Wentz, 1988; MacDonald *et al.* 1991). One may speculate, therefore, if the redistribution phenomenon of progesterone represents a compensatory mechanism designed to overcome the high metabolic clearance rate of the hormone (Braasch *et al.* 1988; O'Malley & Stroitt, 1991). Such compensation would lead to increased progesterone concentrations in the blood and, consequently, could increase the reproductive success of an individual organism, as well as a whole species. It is interesting to note that fatness is associated with fertility (Frisch, 1990). Indeed, a certain minimal amount of body fat appears to be necessary for the regularity of menstrual cycles in humans since irregular cycles and periods of amenorrhoea are observed frequently in women with anorexia nervosa (Warren & Vande Wiele, 1973) and female athletes (Schwartz, Cumming, Riordan, Selye, Yen & Rebar, 1981).

The pharmacological literature contains a few examples of drugs that display peculiar kinetics similar to those reported by us, i.e. the presence of a double peak in blood levels after single doses (Bergstrom, Kay, Harkcom & Wagner, 1981). Of particular interest is a report on the existence of such peaks after oral administration of contraceptive steroids (Zacur, Burkman, Kimball, Kwiterovich & Bell, 1992). No data exist, however, on similar kinetics after drug administration by routes other than oral. In addition, mechanisms responsible for those phenomena are different (Bergstrom *et al.* 1981; Zacur *et al.* 1992).

In summary, we have documented the reappearance of progesterone in the circulation of the monkey after a single injection. Our finding is supported by: (1) parallel reappearance of labelled metabolites; (2) significant association in the redistribution phase between the relative amplitudes of labelled progesterone and its metabolites; (3) mathematical equations that fit the data satisfactorily; and (4) the remarkable constancy of temporal parameters for the reappearance in all trials.

APPENDIX

Explanation of equations

Let c_1 be the concentration of [^{3}H]progesterone in the circulation in the initial phase of distribution and elimination and t be time; let A, B, m and k be equation parameters such that A and B are scale factors (A and B are not equal to zero) and m and k are elimination constants ($m, k > 0$). Let $u(t)$ be the unit step function, taking on the value of zero or unity according to whether its argument is less than or equal to zero or greater than zero. Four cases may be distinguished with regard to the $c_1(t)$. If the hormone administration occurred as *a perfect bolus*, then (Gibaldi & Perrier, 1982; Godfrey, 1983):

for a single compartment and for *two compartments with a single real characteristic function*

$$c_1 = Ae^{-mt}, \tag{A1}$$

or for *two compartments with two real distinct characteristic functions*

$$c_1 = Ae^{-mt} + Be^{-kt}. \tag{A2}$$

If the hormone administration occurred as *an ultra-short infusion* at a constant rate, then (Gibaldi & Perrier, 1982; Godfrey, 1983):

for a *single compartment* and for *two compartments with a single real characteristic function*

$$c_1 = [1 - u(t-r)]A(1-e^{-mt}) + u(t-r)A(1-e^{-mr})e^{-m(t-r)}, \tag{A3}$$

or for *two compartments with two real distinct characteristic functions*

$$c_1 = [1 - u(t-r)][A(1-e^{-mt}) + B(1-e^{-kt})] + u(t-r)[A(1-e^{-mr})e^{-m(t-r)} + B(1-e^{-kr})e^{-k(t-r)}]. \tag{A4}$$

(Equations (A1) and (A3) describe, in fact, concentration in the first compartment of any n-compartmental ($n \geqslant 2$) catenary series with a single real characteristic function (Godfrey, 1983)). If, in the redistribution phase (during the reappearance; Fig. 2), the intrinsic characteristics of the compartmental systems (elimination constants) are the same, then only the scale factors are different since the accumulated amount of [^{3}H]progesterone in the tissues must have been smaller than the administered dose, upon which scale factors depend (Gibaldi & Perrier, 1982). Let $c_2(t)$ denote dependence of the [^{3}H]progesterone concentration in the circulation at a time when scale factors are G and/or H. Then the concentration of [^{3}H]progesterone in circulation in the redistribution phase only is:

$$c_1(t) + \int_0^t c_2(t-\tau)c_1(t)\mathrm{d}\tau,$$

where the second term is a convolution integral (MacDonald, 1978; Godfrey, 1983). The joint analytical form of the model equations for the initial phase and the redistribution phase is:

$$c_1(t) + u(t-r-s)\int_0^t c_2(t-\tau)c_1(t)\mathrm{d}\tau,$$

where s is the time after which the redistribution phase begins in relation to r ($s > 0$) and $r = 0$ in the cases of perfect bolus injection. Let the units of $u(t-r-s)$ be such that the units of scale factors G and H are those of concentration. By applying the convolution theorem to the Laplace transforms of functions (A1)–(A4) for $c_1(t)$ and $c_2(t)$, and by making use of the shift property of the Laplace transforms with regard to time, the final equations for the [^{3}H]progesterone concentration in the circulation in both phases (c) are:

for eqn (A1)
$$c = Ae^{-mt} + u(t-s)AG(t-s)e^{-m(t-s)}, \tag{A5}$$

for eqn (A2)
$$c = Ae^{-mt} + Be^{-kt} + u(t-s)\{AG(t-s)e^{-m(t-s)} + BH(t-s)e^{-k(t-s)} + [(AH+BG)/(m-k)](e^{-k(t-s)} - e^{-m(t-s)})\}, \tag{A6}$$

for eqn (A3)
$$c = [1-u(t-r)]A(1-e^{-mt}) + u(t-r)A(1-e^{-mr})e^{-m(t-r)} + u(t-r-s)A(1-e^{-mr})G(t-r-s)e^{-m(t-r-s)}, \tag{A7}$$

for eqn (A4)
$$\begin{aligned} c = {} & [1-u(t-r)][A(1-e^{-mt}) + B(1-e^{-kt}] + u(t-r)[A(1-e^{-mr})e^{-m(t-r)} + B(1-e^{-kr})e^{-k(t-r)}] \\ & + u(t-r-s)\{A(1-e^{-mr})G(t-r-s)e^{-m(t-r-s)} + B(1-e^{-kr})H(t-r-s)e^{-k(t-r-s)} \\ & + \{[B(1-e^{-kr})G + A(1-e^{-mr})H]/(m-k)\}(e^{-k(t-r-s)} - e^{-m(t-r-s)})\}. \end{aligned} \tag{A8}$$

During fitting of the data to eqns (A5) and (A7), factors AG and $A(1-e^{-mr})G$ were considered as a single parameter.

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