Progesterone Retention by Rat Uterus I: Pharmacokinetics after Uterine Intraluminal Instillation

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Abstract □ Tritium-labeled progesterone was administered to mature female rats in the proestrous stage by three different routes, gastric intubation, subcutaneous injection, and uterine intraluminal instillation, to study the kinetics involved in the uptake and retention of radioactivity by the uterus and various other tissues. Progesterone was retained at a much higher level and for a more prolonged period in the rat uterus after uterine intraluminal instillation. Progesterone bioavailability to the uterus was 45 times higher by uterine intraluminal instillation than by either gastric intubation or subcutaneous injection. Progesterone absorption by the rat endometrium was extremely fast. The observed biphasic decrease of radioactivity from the uterine tissue was explained adequately by a pharmacokinetic model in which progesterone is assumed to be present in two compartments within the uterine tissue. The pharmacokinetic parameters showed that the progesterone biological half-life in the uterine tissue during the α-phase was about 6.5 min while that in the β-phase was about 230 min.

Keyphrases □ Progesterone—tissue distribution, different routes of administration compared □ Distribution, tissue—progesterone, different routes of administration compared □ Pharmacokinetics—progesterone, tissue distribution, different routes of administration compared □ Administration routes—uterine intraluminal instillation compared to gastric intubation and subcutaneous injection, progesterone tissue distribution □ Progestins—progesterone, tissue distribution, different routes of administration compared

The effectiveness of progestins as contraceptives, with or without the addition of estrogens, is well known, as witnessed by the successful application of "the pill" and "the minipill" in fertility control. The pill, which contains combinations of an estrogen and a progestin, exerts its effect through ovulation inhibition. With the minipill, which contains only progestin and in smaller amounts, the exact mechanism involved in the prevention of conception is still not clear but does not necessarily require the concomitant inhibition of ovulation. Evidence suggests that the site of action is probably the uterus, cervix, or fallopian tubes (1). Therefore, progestin may act directly on end target tissue to produce a contraceptive effect without the necessary participation of, or mediation by, the ovary or pituitary glands. If this is the case, the local administration of contraceptive progestins to the uterus logically should be the route of choice. This route would deliver the drug directly to the target tissue, require a much smaller dose than oral tablets, and, consequently, minimize undesirable feedback controls that are inevitable in systemic administrations.

Contraceptive progestins have been impregnated in intrauterine devices to form a sustained-release drug delivery system (2–12). The device[1], also called the "Uterine Progesterone System," releases a constant amount of progesterone at a controlled rate for many months upon placement in the uterine intraluminal cavity.

The present study was designed to investigate the pharmacokinetics involved in the uterine absorption and retention of progesterone administered by direct uterine intraluminal instillation as well as the relative bioavailability of progesterone to the rat uterine tissue upon three different routes of administration: oral intubation, subcutaneous injection, and uterine intraluminal instillation.

EXPERIMENTAL

Reagents and Chemicals—1,2-^{3}H-Progesterone[2] (56 Ci/mmole) was purified by TLC before use. Other chemicals were reagent grade.

Administration of ^{3}H-Progesterone—Mature female Sprague–Dawley rats, 180–250 g, were acclimated in an animal room with a 12-hr day–12-hr night lighting schedule until their estrous cycles had stabilized and resumed regular 4–5-day cycles as determined by vaginal smear cell typing. Tritium-labeled progesterone was administered to separate groups of female rats in proestrous stages by gastric intubation, subcutaneous injection, or direct uterine intraluminal instillation.

In oral administration, the rats were fasted overnight for 16 hr and ^{3}H-progesterone (0.15 μg = 5.26 × 10^6 dpm) in 1 ml of 5% ethanol was administered by gastric intubation. The same amount of ^{3}H-progesterone in 1 ml of 5% ethanol was used in subcutaneous injection. For the uterine intraluminal instillation, ^{3}H-progesterone (either 0.015 or 0.15 μg = 8.26 × 10^6 dpm) was dissolved in 40 μl of 5% ethanol and one-half of the solution (20 μl) was instilled into the luminal cavity of each uterine horn through the vaginal route or laparotomy. The solution was placed approximately at the midpoint of the uterine horn. Earlier tests with the dye solution showed no observable discharge of the injected solution through the uterine cervix using this route. Each experimental point was obtained from the pooled data of three or four rats.

Quantification of Radioactivity in Various Tissues—The rats were killed by cervical dislocation at selected times from 5 min to 16 hr after tritium-labeled progesterone administration. Uterus and other tissues, *i.e.*, liver, brain, diaphragm, muscle, lung, plasma, vaginal wall, and fat, were excised, rinsed with Ringer's solution, and minced with scissors. The minced tissue (0.1–0.5 g) was digested with 2 ml of tissue solubilizer[3] at 55° for 16 hr. After digestion, 0.5 ml of benzoyl peroxide solution (15% in toluene) was added and the mixture was further incubated at 55° for another 30 min.

With the exception of the uterine preparation, aliquots of the digested tissue solution (0.3–1.5 ml) were taken for the measurement of radioactivity. For the uterine tissue, 5 ml of scintillation fluor cocktail was added following the treatment with benzoyl peroxide solution to dissolve the resulting white precipitates. The mixture was shaken, and 1 ml of the solution was taken for the estimation of radioactivity.

To the sample to be counted was added 12 ml of toluene-based scintillation fluor cocktail containing 0.4% (w/v) diphenyloxazole, 0.005% (w/v) 1,4-bis[2-(5-phenyloxazolyl)]benzene, 25% (v/v) nonionic surfactant[4], and 5% (v/v) water. This mixture was then maintained at 4° for at least 16 hr in the dark before it was counted in a liquid scintillation spectrometer[5]. The quenching effect was estimated by means of a preconstructed calibration curve plotted using the external standard channel ratio *versus* counting efficiency.

RESULTS AND DISCUSSION

Distribution of Radioactivity in Various Tissues—After the oral administration of 0.15 μg of tritium-labeled progesterone, most radioactivity was found in the liver throughout the study (Fig. 1). As much as 40% of the radioactivity administered was in the liver 4 hr after admin-

[1] Progestasert, Alza Corp.

[2] New England Nuclear, Boston, Mass.

[3] Protosol, New England Nuclear.

[4] Triton X-100.

[5] Packard model 3385 Tri-Carb liquid scintillation spectrometer.

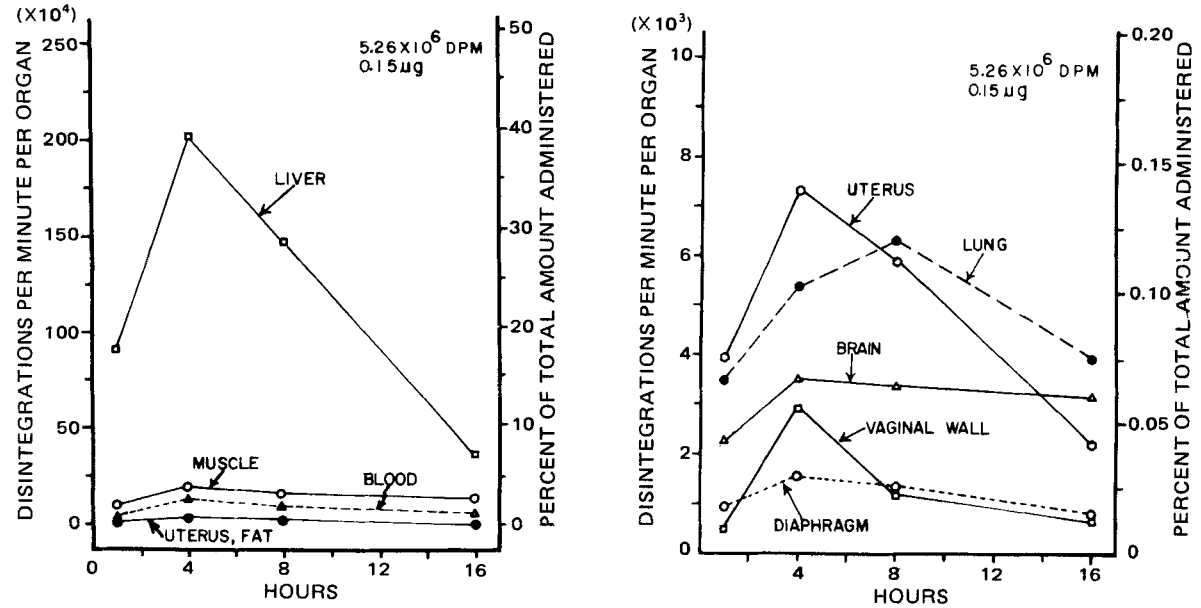


Figure 1—*Retention of radioactivity by various tissues after the gastric intubation of ^3H-progesterone.*

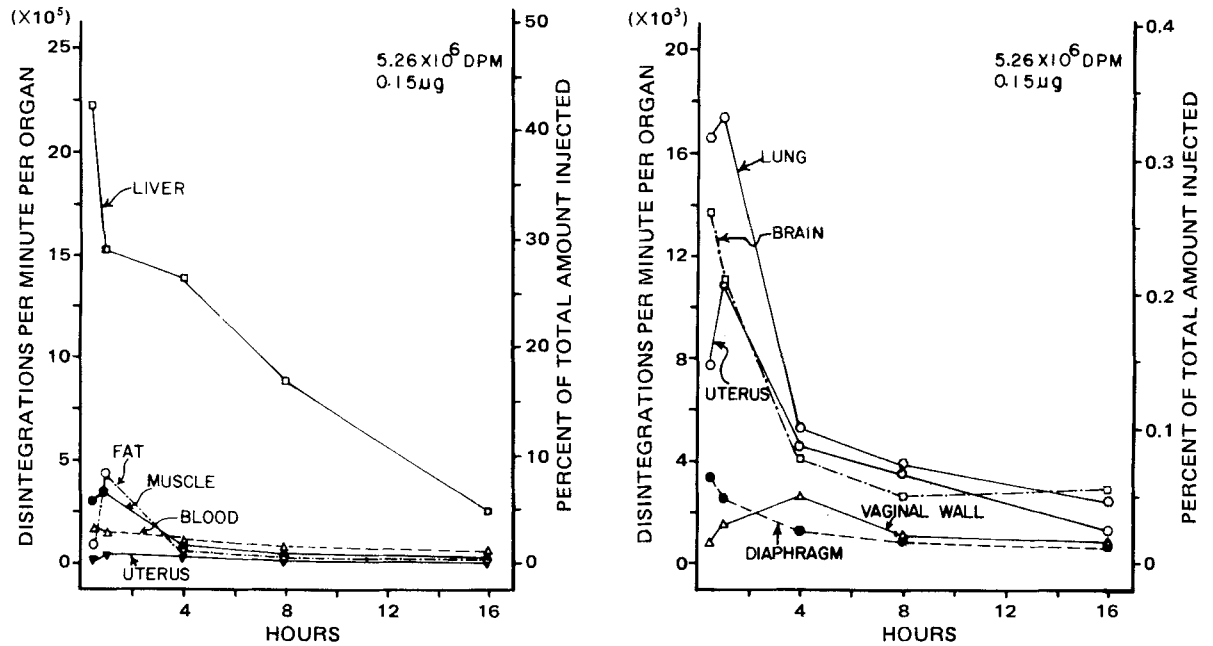


Figure 2—*Retention of radioactivity by various tissues after the subcutaneous injection of ^3H-progesterone.*

istration. In comparison, other organs or tissues, such as the uterus, muscle, blood, fat, lung, brain, diaphragm, and vaginal wall, retained only a fraction of a percent of the administered radioactivity. For instance, a maximum of only 0.15% of the total radioactivity administered was in the uterus tissue 4 hr after gastric intubation.

On a concentration basis expressed as disintegrations per minute per gram of tissue, however, uterus and vaginal wall tissues retained high concentrations of radioactivity for a prolonged period, typical of progesterone target tissues (Table I). The liver, however, retained the highest concentration of radioactivity expressed on a disintegrations per minute per gram of tissue basis. The radioactivity concentration in the liver was about 20 times that of the uterus up to 8 hr after administration.

Table I—Radioactivity Distribution in Rat Tissues after Gastric Intubation of ^3H-Progesterone

	Radioactivity[a], dpm/g of Tissue								
Hours	Uterus	Vaginal Wall	Brain	Liver	Lung	Diaphragm	Thigh Muscle	Fat	Blood
1	5937 ± 1312	2652 ± 375	1446 ± 79	107,063 ± 28,997	3388 ± 416	1774 ± 369	1139 ± 221	725 ± 90	3630 ± 901
4	11,762 ± 1729	19,405 ± 2705	2223 ± 255	255,913 ± 55,401	5227 ± 769	3132 ± 875	2257 ± 727	2057 ± 308	9000 ± 2385
8	10,158 ± 1327	7430 ± 1884	2113 ± 201	181,916 ± 50,085	6148 ± 960	2699 ± 533	2029 ± 351	2265 ± 302	6753 ± 329
16	6387 ± 727	3644 ± 994	2042 ± 227	51,499 ± 6458	3804 ± 575	1656 ± 246	1954 ± 289	1724 ± 361	4460 ± 364

[a] Total ^3H-progesterone administered = 0.15 µg = 5.26 × 10^6 dpm in 1 ml of 5% ethanol. The data pooled from either three or four rats were expressed as mean ± *SD*.

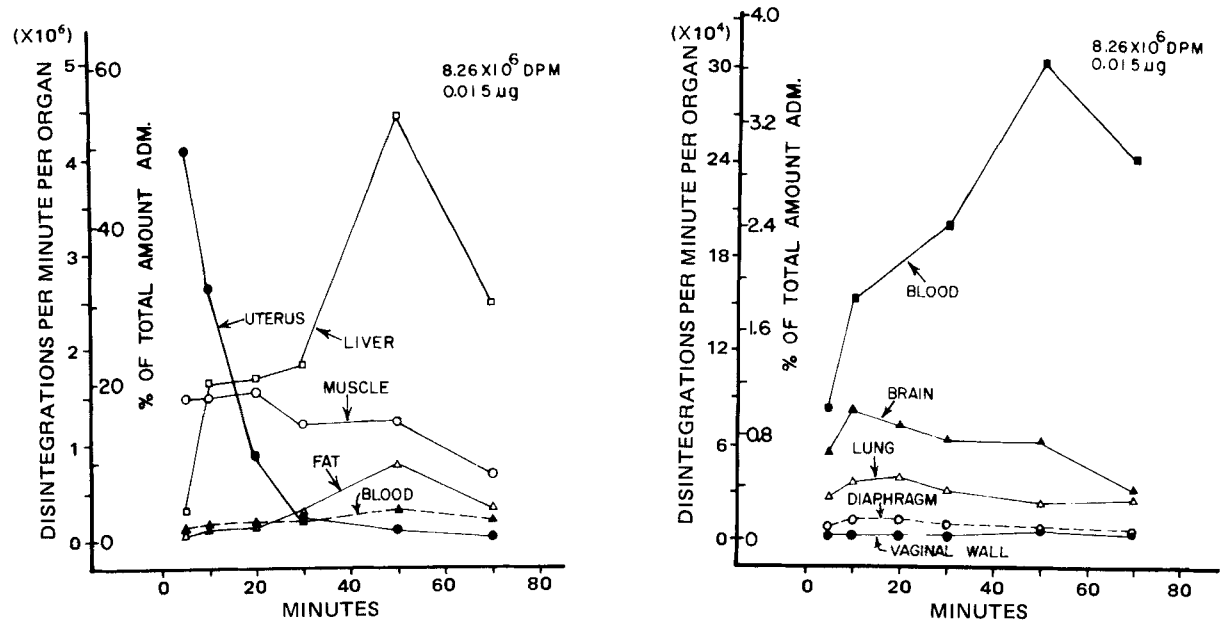


Figure 3—*Retention of radioactivity by various tissues after the uterine intraluminal instillation of ^3H-progesterone.*

A similar pattern and level of radioactivity distribution in various rat tissues were observed when the tritium-labeled progesterone (0.15 μg) was administered by subcutaneous injection (Fig. 2 and Table II). Most radioactivity again was found in the liver. Although as much as 7% of the total dose was in fat tissues 60 min after administration, only a fraction of a percent of the total dose was in other organs and tissues.

Tritium-labeled progesterone (0.15 μg) was also administered by direct instillation into the luminal cavity of rat uterine horns through laparotomy. In an earlier attempt, progesterone solution was introduced into the uterine luminal cavity by the vaginal route according to the method reported by Stone and Martin (13). However, uncertainties always arose with this route regarding the definitive placement of the solutions in two separate uterine horns of a rat and not twice in the same horn. Furthermore, the exact point of placement of the solution along the length of the uterine horn was also rather difficult to ascertain. Therefore, direct injection of the progesterone solution into the luminal cavity of the uterine horn after laparotomy was preferred.

As expected, large amounts of radioactivity were initially found in the uterine tissue after uterine intraluminal instillation (Figs. 3 and 4). The decrease in uterine radioactivity followed a typical biphasic exponential curve: an initial phase of rapid decrease, which lasted for about 30 min, followed by a second phase with a much slower rate of decrease. The decrease in uterine radioactivity coincided with the increases in liver and blood uptake (Fig. 3). The maximum amount of radioactivity in the liver, about 55% of the total dose, was reached 50 min after instillation.

In oral administration, the liver uptake of progesterone did not peak until 4 hr after administration. This result indicates that progesterone absorption is definitely much faster in the uterus than in the GI tract. Although direct comparisons with subcutaneous injection is difficult because the studies were done on different time courses, the rate of systemic availability of progesterone administered by uterine intraluminal instillation is probably faster than that after subcutaneous injection.

As with the other two modes of administration, only a small fraction of administered progesterone reached various other tissues after uterine

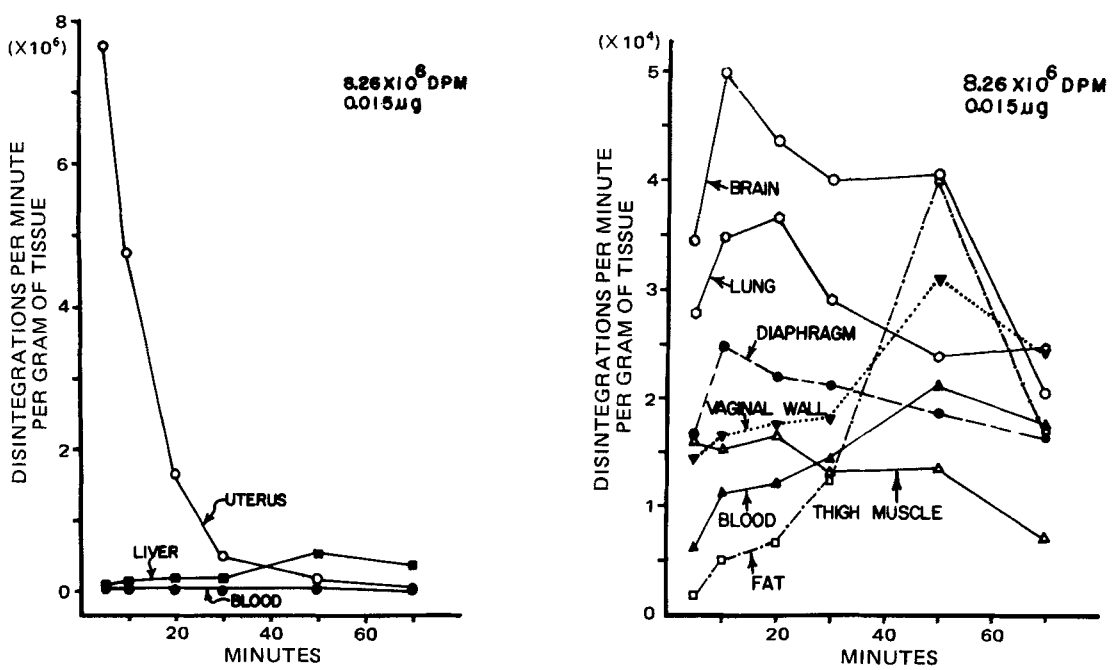


Figure 4—*Radioactivity concentrations in various tissues after the uterine intraluminal instillation of ^3H-progesterone.*

Table II—Radioactivity Distribution in Rat Tissues after Subcutaneous Administration of ^{3}H-Progesterone

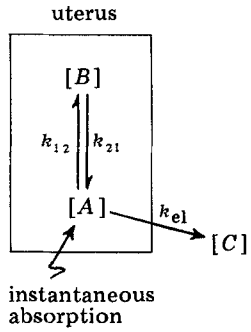
	Radioactivity[a], dpm/g of tissue								
Hours	Uterus	Vaginal Wall	Brain	Liver	Lung	Diaphragm	Thigh Muscle	Fat	Blood
0.5	15,646 ± 4386	5738 ± 688	8684 ± 333	283,431 ± 53,826	16,105 ± 3064	6773 ± 912	2774 ± 60	5533 ± 848	11,837 ± 3050
1	20,455 ± 4627	7973 ± 2161	7107 ± 751	187,986 ± 13,686	16,845 ± 2696	5011 ± 502	2836 ± 896	18,900 ± 3128	9337 ± 1510
4	7539 ± 776	17,920 ± 1248	2585 ± 154	179,073 ± 3830	5111 ± 434	2383 ± 176	1588 ± 35	7619 ± 647	8547 ± 2184
8	5591 ± 756	3339 ± 158	1648 ± 196	109,179 ± 16,957	3719 ± 361	1581 ± 153	1545 ± 181	4584 ± 1026	3753 ± 1067
16	3787 ± 707	3877 ± 1318	1850 ± 284	41,951 ± 9443	2367 ± 1128	1424 ± 10	1314 ± 163	2070 ± 71	3877 ± 329

[a] Total ^{3}H-progesterone administered = 0.15 μg = 5.26 × 10^6 dpm in 1 ml of 5% ethanol. The data pooled from either three or four rats were expressed as mean ± *SD*.

intraluminal instillation (Figs. 3 and 4). Substantially higher concentrations of progesterone, however, were detected in most tissues studied with this mode of administration than those obtained by the other two routes of administration (Tables I and II and Fig. 4). Uterine tissue, as expected, retained a high concentration of radioactivity for a rather long time. Progesterone bioavailability in the uterus, estimated from the area under the radioactivity–time curve, was about 45 times higher by uterine intraluminal instillation than that obtained by either gastric intubation or subcutaneous injection (Figs. 1–3).

Uterine Absorption of Progesterone Administered by Direct Uterine Intraluminal Instillation—The absorption rate of progesterone by uterine tissue was estimated by measuring the radioactivity remaining in the luminal cavity at various times after instillation. Uterine endometrium absorbed progesterone with great rapidity. At a dose of 0.15 μg (in 40 μl of 5% ethanol solution), essentially all radioactivity (95%) was absorbed by the uterine tissue 5 min after instillation. Similar results were obtained with a 0.015-μg dose. Therefore, the rat uterine endometrium is an extremely efficient tissue for progesterone absorption. Similar results in spayed mice were reported (14).

Pharmacokinetic Analysis of Uterine Retention of Progesterone Administered by Uterine Intraluminal Instillation—The amount of radioactivity retained by uterine tissue was determined at different intervals after the instillation of tritium-labeled progesterone into the luminal cavity of the uterus. The radioactivity in the uterine tissue following rapid absorption from the luminal cavity was eliminated in a kinetic pattern closely resembling a biphasic exponential curve (Fig. 5). A plausible pharmacokinetic model to describe this phenomenon is shown in Scheme I, where [*A*] is the progesterone in compartment *A* of the uterus, [*B*] is the progesterone in compartment *B* of the uterus, [*C*] is the amount of progesterone excreted out of the uterus, k_{12} is the rate constant for $A \rightarrow B$, k_{21} is the rate constant for $B \rightarrow A$, and k_{el} is the elimination rate constant.



Scheme I

The derivation of the pertinent pharmacokinetic parameters is given as follows. Let *D* equal the total amount of progesterone administered and V_d equal the apparent volume of distribution. Then:

$$\frac{dA}{dt} = k_{21}B - (k_{12} + k_{el})A \quad \text{(Eq. 1)}$$

$$\frac{dB}{dt} = k_{12}A - k_{21}B \quad \text{(Eq. 2)}$$

$$\frac{dC}{dt} = k_{el}A \quad \text{(Eq. 3)}$$

Solving for *A* and *B* from Eqs. 1–3 yields:

$$A = \frac{D}{\alpha - \beta}[(\alpha - k_{21})e^{-\alpha t} + (k_{21} - \beta)e^{-\beta t}] \quad \text{(Eq. 4)}$$

$$B = \frac{Dk_{12}}{\alpha - \beta}(e^{-\beta t} - e^{-\alpha t}) \quad \text{(Eq. 5)}$$

where:

$$\alpha = \tfrac{1}{2}[(k_{12} + k_{21} + k_{el}) + \sqrt{(k_{12} + k_{21} + k_{el})^2 - 4k_{21}k_{el}}] \quad \text{(Eq. 6)}$$

$$\beta = \tfrac{1}{2}[(k_{12} + k_{21} + k_{el}) - \sqrt{(k_{12} + k_{21} + k_{el})^2 - 4k_{21}k_{el}}] \quad \text{(Eq. 7)}$$

$$A + B = \frac{D}{\alpha - \beta}[(k_{el} - \beta)e^{-\alpha t} + (\alpha - k_{el})e^{-\beta t}] \quad \text{(Eq. 8)}$$

The total concentration of radioactivity in the uterus, C_{ut}, is:

$$C_{ut} = \frac{A + B}{V_d} = \frac{D}{V_d(\alpha - \beta)}[(k_{el} - \beta)e^{-\alpha t} + (\alpha - k_{el})e^{-\beta t}] \quad \text{(Eq. 9)}$$

Let:

$$m_1 = \frac{D(k_{el} - \beta)}{V_d(\alpha - \beta)} \quad \text{(Eq. 10)}$$

and:

$$m_2 = \frac{D(\alpha - k_{el})}{V_d(\alpha - \beta)} \quad \text{(Eq. 11)}$$

Then:

$$C_{ut} = m_1e^{-\alpha t} + m_2e^{-\beta t} \quad \text{(Eq. 12)}$$

where m_1, m_2, α, and β are obtained from graphical analysis of experimental data plotted as log C_{ut} *versus* *t* and:

$$k_{el} = \frac{\alpha m_1 + \beta m_2}{m_1 + m_2} \quad \text{(Eq. 13)}$$

$$k_{21} = \frac{\alpha\beta}{k_{el}} \quad \text{(Eq. 14)}$$

$$k_{12} = \alpha + \beta - k_{21} - k_{el} \quad \text{(Eq. 15)}$$

This particular model is established with the following assumptions and/or approximations:

1. Progesterone is absorbed so rapidly by uterine tissue upon intraluminal instillation that absorption is assumed, for practical purposes, as instantaneous.

2. Upon reaching uterine tissue, progesterone is quickly redistributed into other parts of the body in a first-order kinetic rate with a rate constant of k_{el}, while a simultaneous process also takes place within uterine tissue where progesterone molecules diffuse to a deep compartment within the uterine cell. Progesterone redistribution within the uterine tissue is a reversible process with a forward rate constant of k_{12} and a reverse rate constant of k_{21}.

3. The nonpolar and conjugated metabolites of progesterone in the uterus are all treated as progesterone in this model. This treatment is based on the fact that most nonpolar metabolites are also known progestins, while the conjugates, since they form a reversible equilibration with nonconjugated moieties within the uterine tissue, should be considered equally active physiologically. Since progesterone is proposed to act directly on uterine tissue to cause contraception, for the collective quantitative assessment of the steroid action in uterine tissue, one should pool all these metabolites to achieve a better approximation.

4. Since progesterone is eliminated out of the body not directly *via* the uterus, the reversible influx of progesterone from blood to the uterus is likely to be insignificant. Therefore, the process was omitted for simplification.

The values for β, m_1, α, and m_2 were obtained by feathering analysis of the plot of log disintegrations per minute per gram of tissue *versus* time. The values for k_{el}, k_{12}, and k_{21} are 0.110, 8.27 × 10^{-4}, and 3.09 ×

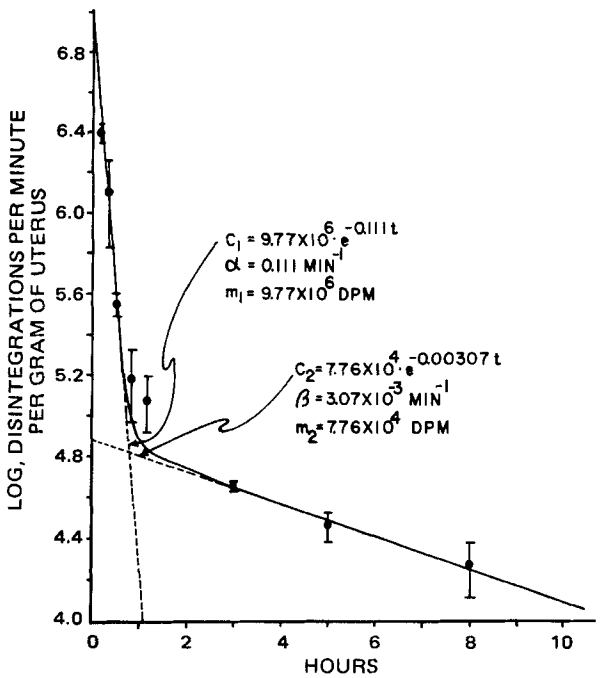


Figure 5—*Pharmacokinetic analysis of the uterine retention of radioactivity after the uterine intraluminal instillation of ^3H-progesterone (0.15 μg = 8.26 × 10^6 dpm). Key: C_2, straight line obtained from the β-phase decay; C_1, straight line obtained by "feathering method"; and solid biphasic line, computer-predicted value for C_{ut} (Eq. 12) based on the obtained pharmacokinetic parameters. The experimental values are presented as mean ± SD.*

10^{-3} min^{-1}, respectively. The computer plot of Eq. 12 is shown in Fig. 5. Except for a few points in the inflection area, the experimental values fit reasonably well with the computer-predicted values derived from the proposed pharmacokinetic model. This model is definitely oversimplified and should not be used to argue the definitive mechanisms involved in the uterine retention of progesterone. The constant obtained from k_{12}/k_{21} is a complex value probably contributed by various mixed factors such as progesterone binding to various subcellular components or the heterogeneous distribution of progesterone between the intracellular and interstitial fluids of the uterine tissue.

Based on these kinetic model analyses, the slope in the α-phase approximates k_{el} while the slope in the β-phase approximates the dissociation constant k_{21}. This result connotes a different physiological significance than the two-compartment open model analysis commonly used in describing drug disappearance from blood where the slope in the β-phase approximates the k_{el} value. Based on the values of k_{el} and k_{21}, the progesterone biological half-life in the uterine tissue was calculated to be 6.5 min in the α-phase and 230 min in the β-phase. In comparison, the progesterone biological half-life in rat peripheral serum was reported to be 18 min in the β-phase (15), which is much shorter than that in the uterus. This result further substantiates the earlier hypothesis that the reverse influx of progesterone from blood to uterus is likely to be insignificant in uterine intraluminal instillation.

To investigate the consequences of the application of a "Uterine Progesterone System," further studies should simulate constant uterine administration of progesterone until a steady state is reached. Progesterone distribution in uterus and various other tissues should then be assessed to compare the relative amount of progesterone that may exist in the uterus against other parts of the body. Since progesterone is likely to exert the contraceptive effect locally on uterine tissue, the ideal steady state after drug administration should exhibit a large amount of drug in the uterus and negligible amounts in other parts of the body. Furthermore, the extent of the uterine retention of progesterone in the steady state assessed by the actual experimental measurement should be compared to the predicted value based on the pharmacokinetic parameters obtained in this study. These and other studies pertinent to the use of the "Uterine Progesterone System" will be published in subsequent papers.

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ACKNOWLEDGMENTS AND ADDRESSES

Received November 10, 1976, from the *Department of Applied Pharmaceutical Sciences and Biopharmaceutical Sciences, College of Pharmacy, University of Utah, Salt Lake City, UT 84112.*

Accepted for publication March 1, 1977.

Supported by Grant HD-09420 from the National Institutes of Health.

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