Characterization of 17β-estradiol-^{3}H Single-Injection Disappearance Curves in Rat Plasma and Red Cells[1] (36602)

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Single intravenous injection of isotopically labeled estrogens has shown two-component plasma disappearance curves (1–4). However, these data represent either the presumption or arbitrary resolution of strict first-order processes over a predetermined period of time. A more sophisticated resolution of estrogen disappearance curves into a finite number of exponential components could lead to a more detailed understanding about metabolism and distribution. In the present study the disappearance of labeled estradiol from the blood in the rat was followed from time of injection to the time when the isotope levels were almost negligible in order to investigate the multicompartment system involved in estrogen clearance from the blood.

Materials and Methods. Mature female rats of the Sprague-Dawley-Rolfsmeyer strain whose body weights ranged from 250 to 350 g were maintained at a constant environmental temperature of 25.6 ± 1° with daily exposure to 14 hr of light and 10 hr of darkness. Each rat estrous cycle was followed daily by vaginal smear for 2 weeks prior to the experiment. Only rats showing a typical diestrous smear were used.

The rats were anesthesized with sodium pentobarbital and a PE50 polyethylene cannula was inserted into the right jugular vein, advanced toward the heart and secured in the right ventricle for serial blood sampling.

A physiological dose of 17β-estradiol (0.01 μg/100 g body wt) was used in all experiments. Each of six rats received an injection into the right femoral vein of 17β-estradiol-6,7-^{3}H in 0.2–0.4 ml of physiological saline containing 3.9 to 5.4 μCi. The tritiated hormone (1.55 μCi/0.01 μg) was obtained from New England Nuclear Corporation and was purified by paper chromatography using the solvent system of benzene:hexane:methanol:water (33:66:80:20, V:V). Serial blood samples were taken from the ventricular cannula at 5, 10, 20, 30, 45, 60, 90, 120, 180, 240 and 360 min after injection. Whole blood (0.3 ml) was placed in a Beckman microfuge tube for centrifugation. A duplicate 0.3 ml of whole blood was pipetted into 10 ml of an extraction mixture of 95% toluene and 5% isoamyl alcohol (5). The 0.3 ml whole blood in the microfuge tubes was spun at 19,000 rpm (8000*g*) for 1 hr in a refrigerated centrifuge to pack the red cells. After centrifugation, the red cells were separated from the plasma by passing a double-edged razor blade through the polyethylene microfuge tube. The red cells and plasma were then dropped into separate vials containing 10 ml of the extraction mixture. The suspensions were shaken mechanically for 1 hr. After a 30 min centrifugation (3000*g*), a 9 ml aliquot of the isoamyl-toluene phase was transferred to a vial containing 10 ml of phosphor.

The scintillator system consisted of 0.6% 2-(4-*tert*-butylphenyl)-5-(4-biphenyl)-1,3,4-oxdiazole (butyl-PBD) in toluene (6). The radioactivity was measured in a Packard Automatic Tricarb Spectrometer with 48% counting efficiency for tritium. A recovery of from 70 to 80% of 17β-estradiol-^{3}H added to samples of whole blood plasma or red cells was achieved by this method. The samples were counted for sufficient time to yield a maximal relative standard error of ±2%.

In order to evaluate the clearance curves, the specific activity of the whole blood, plas-

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ma and red cells was plotted against time (minutes after injection) on a semilogarithmic coordinate. This plot of specific activity curve versus time resulted in curves asymptotic to a straight line. The separate exponential components of the resulting disappearance curves were then subtracted out as straight lines by the standard method of Robertson (7). This was accomplished as follows: the best straight line was fitted by inspection through the terminal $h_3e^{-g_3t}$ portion of the curve and extrapolation to time zero on the Y ordinate, which was designated as h_3. The effect of this component on the total curve was removed by subtraction of the values represented by the extrapolated final part of the curve from the corresponding values of the initial curve SA_t, yielding the curve marked first subtraction. Extrapolation of the first subtraction curve to its zero intercept on the Y ordinate gave a second straight-line component $h_2e^{-g_2t}$. By subtraction of this component from the curvilinear part of the first subtraction curve, the straight-line $h_1e^{-g_1t}$, marked second subtraction, was obtained.

The least squared method was used to calculate the regression of radioactivity on time. The standard error was calculated for means of groups by routine statistical methods. Analysis of variance was performed to partition the treatment variation. Treatment mean squares were then compared by the variance ratio, F. Treatments with significant F ratios were separated further by the least significant difference (LSD) criterion or paired Student's t test with unequal variance. All tests were made at the 0.05 level of probability. The above statistical procedures were performed as outlined by Snedecor and Cochran (8).

Results. The logarithm of radioactivity in whole blood was plotted against time over the entire collection period of 360 min. Observation of the curve indicated that three different regression lines could be discerned (Fig. 1). Therefore, the regression equation for an exponential disappearance representative of a three-component model was solved using the method of least-squares analysis. Solutions were obtained by computer for the equation, $SA_t = h_1e^{-g_1t} + h_2e^{-g_2t} + h_3e^{-g_3t}$, where SA was specific radioactivity, g_1 was the slope and h_1 the intercept of the first component of the curve, g_2 and h_2 those for the second component, and g_3 and h_3 those for the third component. Numerical solutions to the equations describing the disappearance of 17β-

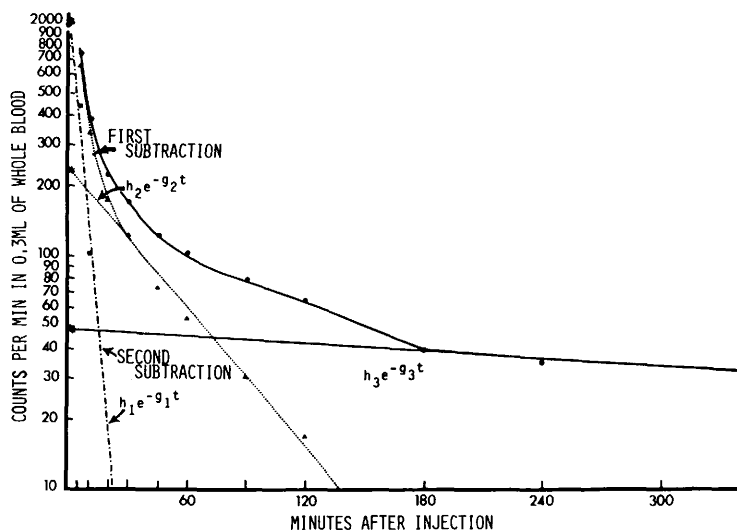

FIG. 1. Analysis of the curve describing regression of whole blood radioactivity following intravenous administration of 0.01 μg 17β-estradiol-6,7-^{3}H (1.55 μCi/100 g body wt) to a female rat during diestrus.

TABLE I. Terms of Equations Describing Disappearance of 17β-Estradiol-^{3}H from Whole Blood, Plasma and Red Cells after Intravenous Injection.

Blood	Curve	$h_1e^{-g_1t}$			$h_2e^{-g_2t}$			$h_3e^{-g_3t}$		
compartments	components:	g_1 (min^{-1})	h_1 (cpm)	$t_{1/2}$ (min)	g_2 (min^{-1})	h_2 (cpm)	$t_{1/2}$ (min)	g_3 (min^{-1})	h_3 (cpm)	$t_{1/2}$ (min)
Whole blood										
Mean		113	813	5.53	22.8	377	30.9	1.16	66.6	718
SE		25	156	1.20	1.3	55	1.9	0.26	11.8	130
Plasma										
Mean		93.2	398	6.48	23.1	290	30.6	1.32	34.9	528
SE		21.0	63	1.22	1.7	39	2.4	0.05	5.7	18
Red cells										
Mean		137	254	4.56	23.4	103	34.7	1.15	17.6	760
SE		33	74	0.47	5.2	29	4.8	0.33	1.8	108

estradiol-^{3}H from whole blood, red blood cells, or plasma are summarized in Table I.

Analysis of variance and then a paired t test indicated that the regressions, intercepts and half-lives derived from the three components of the curve were significantly different from each other ($p < .05$). However, values of the same component for whole blood, red cells, or plasma did not vary significantly.

Two possible models of three compartments each were postulated for the kinetics of 17β-estradiol in the body. These are presented in Fig. 2 and are based upon the theoretical models described by Skinner *et al.* (9). In these models we may designate pool 1 as the blood, which was the site of labeled hormone injection. The fractional rate of estradiol leaving pool 1 and entering pool 2 is represented by k_{21}, while the rate of estradiol returning to pool 1 from pool 2 is fractional rates of estradiol irreversibly leaving pools 2 and 3, respectively.

Based upon the 17β-estradiol disappearance curves, it was evident from the small amount of radioactivity in the blood at the end of 5 min that a considerable amount of the labeled material left the blood before mixing was complete. In order to generate estimates for pools 2 and 3, the size of driving pool 1 (the blood) was calculated using a plasma volume of 4.59 ml/100 g body weight (10). The size of the pools was determined according to Skinner *et al.* (9). A summary of the estimates of pool sizes for models I and II is presented in Table II. Analysis of variance using a factorial design showed a significant F ratio only for turnover rate constants (k_{ij}) in both models. Separation of the significant main effect by Fisher's least significant difference (LSD) criterion showed that in model I, k_{03} and k_{23} were not significantly different from each other, nor were k_{12} and k_{32}. This analysis on model II, solution 1 showed that k_{31}, k_{21} and k_{13} were equal to each other and k_{02} was equal to k_{12}. In model II, solution 2 there were no significant differences in the following comparisons: k_{31} and k_{02}, k_{31} and k_{12}, and k_{03} and k_{13}. Factorial analysis of variance and LSD separation of the significant main effects for the

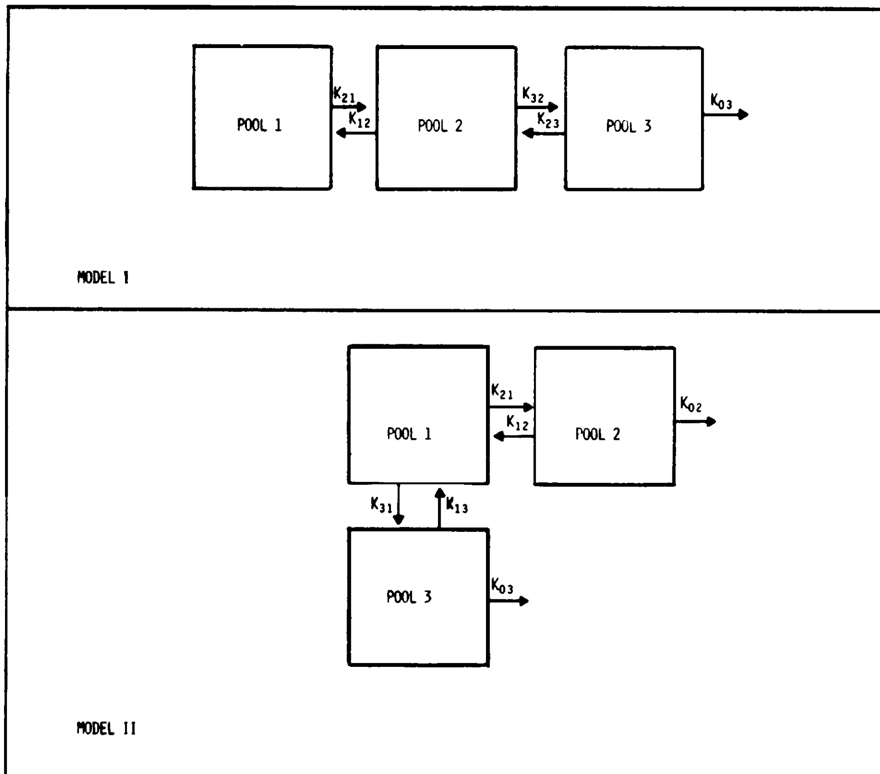

FIG. 2. Possible three-pool models describing 17β-estradiol-6,7-^{3}H disappearance from the blood.

second pool (P_2) and the third pool (P_3) showed that percentage body weight was essentially the same whether calculated from results of whole blood, plasma or red cells.

The first pool (P_1) had a very rapid phase of disappearance with a half-life of 5.5, 6.5 or 4.6 min as measured in whole blood, plasma or red cells, respectively. It is suggested that this represents the physical process of mixing within the blood. P_2 had half-lives of 30.9, 30.6, and 34.6 min, respectively, and represented equilibrium between the blood and another body compartment. P_3 was characterized by long half-lives of 718.4, 527.6, and 757.8 min, respectively, for blood, plasma and red cells (Table I). This outer pool probably represents movement of metabolites of 17β-estradiol.

Discussion. The proposed three-pool model I suggests that estradiol is secreted into blood and becomes rapidly equilibrated in a compartment approximately the size of the extracellular fluid volume. This concept is consistent with that advanced by Short and Rowell (11) for progesterone metabolism. From this rapidly equilibrating pool, it then further equilibrates with a large outer pool of from 30 to 60% of body weight. The third pool may be characterized as that compartment of tissues which incorporate, utilize, and degrade estradiol. A three-pool model of similar kinetic parameters has been described for insulin by Silvers *et al.* (12).

Estimates for model II, solution 1 require a somewhat different interpretation. Mathematical solution of this model indicated that there was a very large pool of 62% of body weight in rapid equilibrium with blood. This pool approximates total body water. P_3 estimates using this model were very small, being only 5% of body weight. The interpretation is that estradiol secreted into blood becomes rapidly equilibrated into total body water including intracellular water of target

TABLE II. Turnover Constants (k) Among Pools and Pool Sizes of Proposed Models Shown in Fig. 2.

Blood compartments	min^{-1} × 10^{-3}								% Body wt	
	k_{32}	k_{31}	k_{23}	k_{21}	k_{13}	k_{12}	k_{03}	k_{02}	Pool 2	Pool 3
					Model I					
Whole blood										
Mean	28.4		6.57	86.7		20.1	1.68		16.3	60.0
SE	6.4		1.74	23.6		4.5	0.42		2.5	14.3
Plasma										
Mean	26.2		4.53	66.4		19.6	5.86		17.1	41.2
SE	4.8		0.86	17.1		5.4	4.24		6.6	9.5
Red cells										
Mean	18.0		4.26	112.5		25.9	1.53		18.8	53.2
SE	2.1		0.44	29.0		4.9	0.42		4.7	8.9
					Model II,[a] Solution 1					
Whole blood										
Mean		39.8		46.9	47.8	2.24	18.3	1.28	65.8	3.61
SE		1.6		8.1	3.1	0.02	3.6	0.29	13.1	1.49
Plasma										
Mean		34.7		39.7	53.9	1.78	22.8	1.42	57.0	4.28
SE		14.0		5.2	2.8	0.14	3.0	0.07	6.1	1.36
Red cells										
Mean		59.4		53.1	46.0	2.30	10.6	1.27	64.2	6.01
SE		15.4		14.9	5.8	0.17	1.5	0.36	10.0	1.89
					Model II,[a] Solution 2					
Whole blood										
Mean		30.8		55.9	3.53	29.5	18.7	18.7	5.27	44.1
SE		5.8		18.3	0.46	6.3	3.5	3.5	1.69	10.1
Plasma										
Mean		22.0		52.8	3.20	31.9	1.42	21.9	4.43	31.5
SE		3.0		16.3	0.17	5.3	0.06	3.5	1.36	3.3
Red cells										
Mean		31.7		80.8	3.57	35.4	1.27	10.6	8.55	40.3
SE		4.7		24.6	0.37	6.7	0.36	1.5	3.08	2.9

[a] Solutions to model II represent the limiting values of solution c, using Skinner's methodology (9).

tissues and also tissues ordinarily considered nonresponsive to estrogens. There is then a gradual recycling of estradiol and metabolites back into the circulation.

The k_{ij}'s are the fractional turnover rates denoting passage from pool j to pool i. The values obtained may aid in justifying the model. The k_{21} shows that pool 1 has a high rate of transfer into pool 2. The relatively high values of k_{21} could indicate the pool contains a metabolizing organ such as the liver.

Results of this study of sampling over a 6-hr period indicate that estradiol utilization may be represented by a complex three-pool system. Furthermore, if pool 2 is representative of the dynamic reservoir for tissue utilization, then the half-life for estradiol in the rat is approximately 30 min, and this may be used as one parameter in measuring the secretion rate.

Summary. Tritiated 17β-estradiol was injected intracardially into rats and blood samples were collected over the succeeding 6-hr

period. Subjection of data on radioactivity to a semilog plot revealed that three regression lines could be developed. By mathematical computation the regressions and intercepts on the *Y*-axis showed that a three-pool model fit the data. Three possible solutions of three-pool models were developed. The most acceptable of these on physiological grounds was model I, in which the first pool represented blood and the half-life of approximately 5 min represented mixing, the second pool represented extracellular fluid of the body and the half-life of approximately 30 min represented the utilization rate of estradiol by target tissues, and the third pool represented the major part of the body fluid and the half-life of approximately 700 min represented the movement of metabolites to the blood and then eliminated via an excretory pathway.

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