Estrone Sulfate: Production Rate and Metabolism in Man

HENRY J. RUDER, LYNN LORIAUX, and M. B. LIPSETT

From the Reproduction Research Branch, National Institute of Child Health and Human Development, Bethesda, Maryland 20014

ABSTRACT Since estrone sulfate (E_1S) is present at high concentration in plasma, we have examined the parameters of the plasma estrone, estradiol, E_1S system. The metabolic clearance rate of E_1S was 157 liter/day (range 70–292) in men and women. Estimated plasma production rates of E_1S were (μgrams per day): men, 77; women, early follicular phase, 95; women, early luteal phase, 182. The conversion of plasma estrone and estradiol to E_1S was measured and from these data and the metabolic clearance rates of the estrogens, the transfer factors were $\rho^{E_1E_1S} = 0.54$ and $\rho^{E_2E_1S} = 0.65$. Using average production rates, all plasma E_1S could be shown to be derived from plasma estrone and estradiol.

The conversion of plasma E_1S to plasma estrone and estradiol was studied. The calculated transfer factors were: $\rho^{E_1SE_1} = 0.21$, $\rho^{E_1SE_2} = 0.014$. Essentially, similar data were obtained when E_1S was given by mouth to two subjects.

We conclude: (*a*) E_1S is a major circulating plasma estrogen and has a long plasma half-life; (*b*) the large contributions of estrone and estradiol to plasma E_1S are more than sufficient to account for all the circulating plasma E_1S.

INTRODUCTION

Estrone-3-sulfate (E_1S)[1] is the major component of the conjugated equine estrogens and of other estrogenic preparations and has been used for over 30 years for treatment of the postmenopausal woman. Although estrone sulfate has been identified in human urine, little is known of its blood levels and metabolism. Twombly and Levitz showed that estrone sulfate had a long half-life in blood (1), and Purdy, Engel, and Oncley (2) found that it was the major plasma metabolite of estradiol and that it was present in high concentration in pregnancy. During previous studies of estrogen metabolism we had postulated (3) that there was another plasma estrogen compartment in equilibrium with plasma estrone (E_1) and estradiol (E_2). Since it seemed probable that this compartment was estrone sulfate, we initiated studies of the origin, rates of metabolism, blood levels, and conversions of estrone sulfate. These studies have confirmed the finding that E_1S is an important plasma metabolite of estradiol and have defined the production rate of E_1S and its interconversions with estrone and estradiol.

Received for publication 24 September 1971 and in revised form 22 November 1971.

[1] *Abbreviations used in this paper:* DCC, dicyclohexylcarbodiimide; DHS, dehydroepiandrosterone sulfate; DMF, dimethyl formamide; E_1, estone; E_2, estradiol; E_1S, estrone-3-sulfate; MCR, metabolic clearance rate; RBC, red blood cells; TLC, thin-layer chromatography.

METHODS

Materials. Estrone-6,-7-^{3}H, estradiol-17β-6,7-^{3}H, estrone-3-NH_4-sulfate-6,-7-^{3}S (40 Ci/mmole), and estrone-4-^{14}C and estradiol-17β-4-^{14}C (45 mCi/mmole) were purchased from New England Nuclear Corp., Boston, Mass. Portions of ^{3}H- and ^{14}C-labeled steroids were combined and radiochemical purity demonstrated before use by constancy of ^{3}H : ^{14}C ratio through a series of derivatives as shown in Fig. 1. Estrone-3-NH_4-sulfate-4-^{14}C (45 mCi/mmole) was prepared from estrone-4-^{14}C (45 mCi/mmole) by a modification of the method of Mumma, Hoiberg, and Weber (4). Estrone-4-^{14}C was dried in a 1 ml centrifuge tube and redissolved in 20 μl of dimethyl formamide (DMF) and chilled to 0°C in an ice-water bath. To the estrone in DMF, 10 μl of chilled 14% concentrated sulfuric acid in DMF (v/v) was added and mixed quickly with a vortex mixer. This was followed by addition of 25 μl of 3 M dicyclohexylcarbodiimide (DCC) in DMF prepared by adding 0.150 g DCC to 0.0240 ml DMF. After mixing, the solution turned a thick white, and was then incubated at 0°C for an additional 15 min. 1 ml of 1 M NH_4OH in methanol was added, the solution mixed, and centrifuged. The supernatant was chromatographed by thin-layer chromatography (TLC) (5) to separate monosulfate from free steroids.

Radiochemical purity of estrone-3-sulfate-4-^{14}C was demonstrated by mixing it with portions of estrone-3-NH_4-sulfate-6,7-^{3}H and demonstrating constancy of ^{3}H : ^{14}C ratios through TLC, isolation, solvolysis of E_1S, and recrystallization of estrone to constant specific activity. Before any study involving the use of estrone sulfate-^{3}H, the steroid was dissolved in sterile saline and preextracted with ether to re-

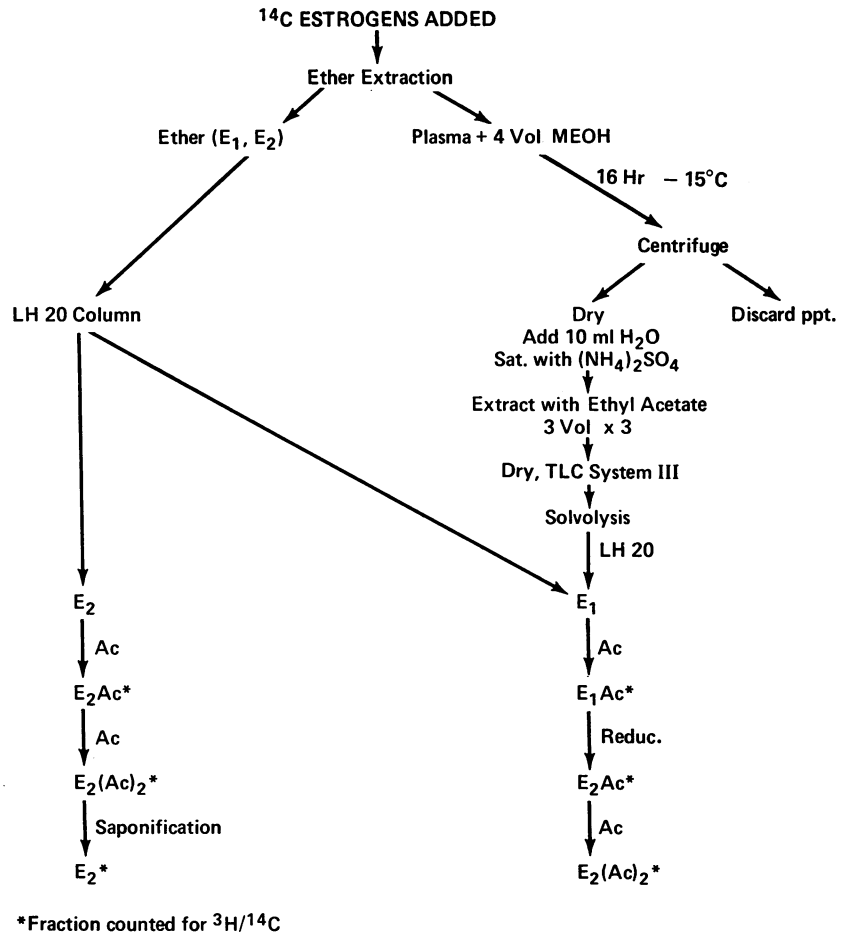

FIGURE 1 Flow chart for measurement of tritium in plasma estrone, estradiol, and E_1S during infusion of ^3H estrogens.

move any contaminating free estrogen. The ether phase was discarded, excess ether removed by an air stream, and portions taken in triplicate for determination of E_1S-^3H. An appropriate volume of sterile saline was then drawn up in a glass syringe for injection or addition to an infusion bottle.

Chromatoquality estrone and estradiol (Calbiochem, Los Angeles, Calif.) were used without further recrystallization. Solvents were spectroquality and used without further purification. TLC was performed on Merck precoated silica gel GF-254 20 × 20 cm glass plates (Merck Chemical Division, Merck & Co., Inc., Rahway, N. J.). The systems used for separations of various steroid and the R_f values are previously described (3, 5, 6). Column chromatography was performed on small Sephadex LH-20 columns (Pharmacia Fine Chemicals Inc., Piscataway, N. J.) which separate E_1 and E_2 as described previously (5).

Subjects. Normal subjects, (Nos. 2–18), ages 18–30 yr were hospitalized at the National Institutes of Health and received no medications. Each woman had normal cyclic menses. Subject 16 was a 28 year old euthyroid subject with a benign thyroid nodule. Subject 1 was a normal man, age 48, in good health.

Clearance rates. Determinations of metabolic clearance rate (MCR) were started at 7:30 a.m. with subjects remaining in the basal state until the end of the study. The infusion techniques were performed as described previously (7).

All short infusions were begun with a 10 μCi bolus given i.v. over 1 min, 30 min later an infusion of approximately 10 μCi/hr was begun and maintained at a constant rate until the end of the study. The duration of the short infusions was usually 165 min from the time of the bolus and varied from 135 min in earlier studies to 210 min in later studies, 30–40 ml of blood was drawn at various times after the bolus was given; the heparinized plasma was separated by centrifugation and stored at −16°C until extracted.

To measure the MCR of E_1S, 20–40 μci of E_1S-^3H was injected over 1 min at 9:00 a.m. via an antecubital vein. 10-ml blood samples were taken at ½, 1, 2, 4, 6, and 8 hr and 20–35-ml blood samples were taken at 11, 14, 19, and 24 hr. Blood was heparinized, centrifuged immediately, and the plasma frozen at −15°C until extracted. Subjects were kept at bed rest with bathroom privileges and fed light meals as desired for the duration of the study except for subjects 1 and 2, who were allowed normal daily activities.

Calculations. The symbols and calculations for the several parameters of the system are essentially those used by Horton and Tait (8). Since all measurements were made in

TABLE I

Metabolic Clearance Rates—Estrone-3-Sulfate-6, 7-^{3}H—Bolus Injections

Subject	Sex and age	Estrone-3-sulfate-^{3}H	Plasma levels of estrone-3-sulfate-6, 7-^{3}H				Y intercept	Volume of distribution	γ (slope)	MCR	MCR_{E_1S}
			0.5–1.0 hr	2.0–5.0 hr	6.0–11 hr	14–24 hr					
		cpm × 10^{-7}	*cpm/liter* × 10^{-4}				*cpm/liter* × 10^{-4}	*liter*	hr^{-1}	*liter/day*	*liter/day/m*
1	M 48	1.12	26.2 (0.5)*	16.7 (2)	14.5 (6)	3.69 (14)	28.1	39.9	0.149	143	76.3
			20.4 (1)	17.8 (3)	8.49 (9)	0.68 (24)					
				17.0 (4)							
2	M 29	1.12	17.3 (0.5)	13.1 (2)	8,55 (6)	3.16 (14)	21.6	51.9	0.158	197	95.7
			15.4 (1)	13.6 (3)	8.87 (9)	0.35 (24)					
				11.8 (4)							
3	M 30	1.55	19.3 (0.5)	11.5 (2)	10.3 (6)	2.32 (14)	16.7	93.4	0.131	292	163
			14.6 (1)	8.2 (4)	3.0 (11)	1.53 (19)					
						0.78 (24)					
4	M-21	1.54	27.3 (1)	28.3 (2.25)	23.4 (6)	13.4 (14)	30.9	49.8	0.0762	91.1	46.8
				19.3 (4.25)	15.8 (8)	7.72 (19)					
					11.3 (11)	4.46 (24)					
5	M 21	1.54		20.4 (2.25)	17.2 (6)	10.4 (14)	22.3	69.1	0.0679	113	55.4
				13.2 (4.25)	11.9 (8)	4.95 (19)					
					11.7 (11)	4.53 (24)					
Mean ±SEM (No. 1–5)	Male	—	—	—	—	—	—	60.8 ±9.4	0.116 ±0.019	167 ±36	87.4 ±21
6	F 18	2.07	39.7 (1)	33.9 (2)	16.0 (6)		47.8	43.3	0.199	207	141
				18.5 (4)	9.6 (8)						
7	F 18	1.43	45.6 (1)	47.7 (2)	26.7 (6)	12.7 (14)	48.8	29.3	0.0913	64.2	39.4
				34.8 (4)	20.9 (8)	8.09 (19)					
					16.7 (11)	6.34 (24)					
8	F 18	1.43	72.9 (1)	61.9 (2)	25.7 (6)	10.4 (14)	72.2	19.8	0.147	69.9	44.5
				31.0 (4)	22.3 (8)	4.05 (19)					
					14.1 (11)	2.21 (24)					
9	F 22	1.47	35.6 (0.33)	18.6 (3)		3.49 (14.5)	27.1	54.2	0.132	172	116
			26.0 (0.75)	12.6 (5)	8.14 (7.5)	1.99 (20)					
			22.3 (1.5)		5.19 (11)	1.44 (24)					
10	F 21	1.47	32.6 (1.5)	22.0 (3)	5.96 (7.5)	1.39 (14.5)	34.5	42.6	0.219	224	134
				8.2 (5)	2.99 (11)	0.29 (20)					
						0.29 (24)					
Mean ±SEM (No. 6–10)	Female	—	—	—	—	—	—	37.8 ±6.0	0.158 ±0.023	146 ±34	94.1 ±22

* Numbers in parentheses are times of collection.

plasma, the compartment is not indicated and the superscript indicates the steroids. Production rate equals MCR × plasma concentration. The conversion ratio, $C^{E_1E_1S}$, is the ratio of radioactivity of product E_1S, to precursor, E_1, when equilibrium has been reached. The transfer factor, $\rho^{E_1E_1S} = C^{E_1E_1S} \times MCR_{E_1S}/MCR_{E_1}$, is the fraction of plasma E_1 production rate converted to plasma E_1S.

Isolation of labeled estrogens. All plasma samples were processed within 3 wk of collection and were treated identically, as shown in Fig. 1. To monitor recovery appropriate amounts (100–1000 cpm) of ^{14}C estrogens were added to each plasma sample. All samples from a single study were processed together. After alkalinizing with 4 drops of NH_4OH, the plasma was extracted three times with 2 vol of ether. The ether extracts containing the neutral steroids were under air and stored until further processing in 5 ml of absolute ethanol at −15°C. Previous tests had shown that only 1–2% of estrone sulfate is removed by the ether, probably in the small amount of water soluble in the ether. Estrone sulfate proved stable in the cold so that any contaminating E_1S was separated from free estrogens by the subsequent column chromatography. The ether-extracted plasma containing the conjugated steroids, including E_1S, was warmed to 40°C and the residual ether removed under an air stream. Recovery of estrone sulfate was found to be inversely related to the quantity of ether remaining at this point. To the plasma was then added 4 vol of absolute methanol to precipitate proteins. Each sample was mixed by shaking for 30 sec and stored at −15°C for at least 16 hr to precipitate lipids. Centrifugation and decanting of the supernatant yielded a yellow methanol solution which contained about 80% of the starting E_1S. After addition of 4 more drops of NH_4OH, samples could be stored indefinitely in the cold until further processing.

Processing E_1S. After 16 hr at −15°C, the methanol-precipitated plasma was centrifuged in 100-ml centrifuge tubes at 2000 *g* for 20 min. The yellow supernatant was decanted and either stored as described above or processed. The supernatant was dried under vacuum until only 2–5 ml of cloudy yellow water remained. This was quantitatively transferred to a 40 ml glass-stoppered tube (final vol. = 10 ml H_2O), alkalinized with 5 drops of concentrated NH_4OH, saturated with dry $(NH_4)_2SO_4$, and extracted three times with 2 vol of ethyl acetate. The ethyl acetate quantitatively removed the E_1S. Recovery to this point is 60–70%. The ethyl acetate was combined and reduced in volume, and the residue was spotted onto TLC plates and the monosulfate fraction isolated (5). Dehydroepandrosterone sulfate (DHS) was used as a marker on both sides of the plate and the DHS identified by streaking with Allen's reagent. After elution from the TLC plate, the samples were dried and then resuspended in 0.2 ml of absolute ethanol. Solvolysis was performed by addition of 5 ml of 10% glacial acetic acid in ethyl acetate and incubation in a tightly capped tube for 16 hr at 50°C. The solvents were removed under N_2 at 40°C. No washing was necessary, and previous tests demonstrated that the estrone was not altered by this procedure. From this point on, derivative formation and portioning for determination of $^3H : ^{14}C$ ratios is as previously described for estrone (6). Recovery of E_1S-^{14}C to the isolation of estrone averaged 50%. In sample studies this procedure was shown to separate completely E_1 and E_1S in plasma since addition of a 100-fold excess of E_1-^3H did not influence the values obtained for tritiated E_1S; and conversely, addition of 100-fold excess of E_1S-^3H did not influence the values obtained for tritiated E_1.

Processing E_1 and E_2. Sample tubes containing neutral steroids in ethanol were dried under N_2 at 40°C and estrone and estradiol separated by column chromatography on Sephadex LH-20 (5). To the appropriate fraction was added 200 μg estrone or estradiol, and derivative formation carried out as shown on Fig. 1 and as previously described

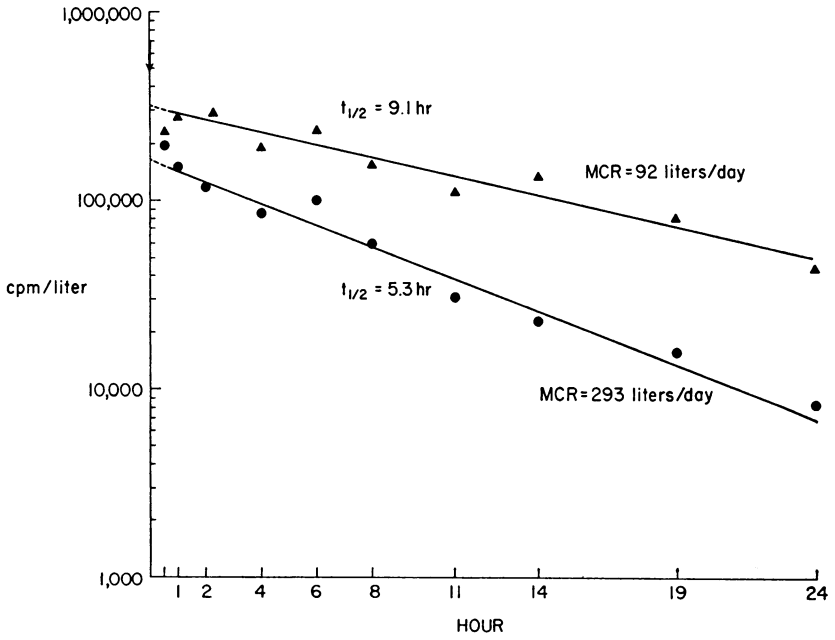

FIGURE 2 Representative data from a low and high MCR_{E_1S}. Infusion started at time 0. The points are the data points; the lines were determined by a computer program.

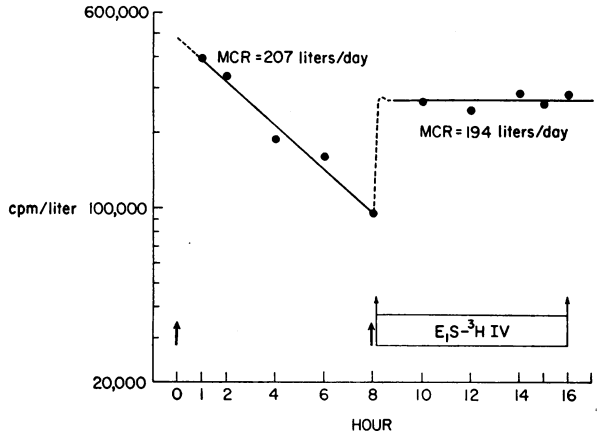

FIGURE 3 MCR_{E_1S} measured after injection of E_1S-^3H at time 0 and at 8 hr followed then by a continuous infusion of E_1S-^3H (subject 6).

(3, 6). After the first four studies were completed, it was evident that in no case did $^3H:^{14}C$ ratios change significantly after the first derivative was isolated, and that in all cases the $^3H:^{14}C$ ratio of the first derivative was the same as that of the final derivative. Hence, thereafter, only two derivatives were counted; the second was the same as the first in the remainder of the studies. In no instance was fewer than 15 cpm above background of ^{14}C present in the second derivative, and except in the case of the E_2 levels during E_1S infusions where conversion to E_2 was quite low, the $^3H:^{14}C$ ratio was greater than 1.0 and less than 8.0 in all samples.

Estrone sulfate binding to red blood cells (RBC). Portions of packed RBC taken during E_1S-^3H infusions were examined for binding of E_1S. We found little (< 5%) of the E_1S-^3H present in whole blood to be associated with RBC.

Counting methods. Radioactivity was measured as previously described (3, 6). All samples were counted to either a minimum of 3000 counts in the carbon channel or for 100 min. Samples were spot checked for differences in quenching and none were found.

Plasma estrogen concentrations. Methods for measurement of plasma concentration of E_1, E_2, and E_1S have been described (5) and normal values for our laboratory are shown in Table III. These values include all patients used in the infusion studies and agree well with those obtained by others (9–11).

Plasma concentration of estradiol monosulfate. To determine if estradiol monosulfate was an important metabolite of estradiol or if it was present in plasma in significant amounts, the following studies were performed. (*a*) We attempted to isolate E_2S-^3H from plasma obtained from two subjects during E_2-^3H infusion. The entire monosulfate fraction was solvolyzed and the E_1 and E_2 fractions isolated. Radiochemical purity was demonstrated only for E_1-^3H, but tritium present in the E_2-^3H fraction was approximately 10% that present as E_1-^3H. Hence conversion of E_2 to E_2S could occur maximally at only $\frac{1}{10}$ the rate of E_2 conversion to E_1S. (*b*) 15 random plasma samples were examined for E_2 monosulfate. No recovery trace was available for E_2-^3H-3-sulfate or E_2-^3H-17-sulfate, but recovery was assumed to be similar to that for E_1S determined simultaneously. After solvolysis of the monosulfate fraction from TLC, both E_1 and E_2 were isolated on Sephadex LH-20. The E_2 fractions were assayed and found to be indistinguishable from blank values (25–50 pg). Assuming the same recovery for E_2 monosulfate as for E_1S, this would mean that E_2S was present at less than 100–150 pg/ml plasma whereas E_1S was measured at concentrations of 250–1000 pg/ml.

RESULTS

E_1S metabolic clearance rates. The data for the disappearance of E_1S-^3H from plasma are shown in Table I. When the data for each subject were analyzed, it was found that in all cases, disappearance curves could be fitted by a single straight line, consistent with a one-compartment system described by a single *Y* intercept (volume of distribution) and a single slope (fractional pool turnover rate). Two representative studies are shown in Fig. 2. The lines of best fit were calculated by computer program, and the points shown are the data points. Metabolic clearance rates were calculated, assuming a one-compartment model, by the formula $MCR = V\gamma$, where V = the volume of distribution and γ = fractional pool turnover rate per unit

TABLE II

Plasma Concentrations and Production Rates (PR) of Estrone, Estradiol, and Estrone-3-Sulfate

	E_1S MCR	E_1S	E_1S PR	E_1 MCR*	E_1	E_1 PR	E_2 MCR*	E_2	E_2 PR
	liter/day	*pg/ml*	*μg/day*	*liter/day*	*pg/ml*	*μg/day*	*liter/day*	*pg/ml*	*μg/day*
Men	167	460	77	2380 (1177)‡	47	112	1700 (892)	34	58
Women Follicular phase	146	654	95	1750 (1070)	62	109	1055 (642)	110	116
Women Luteal phase	146	1,246	182	1750	86	151	1055	193	204

* Average MCR for this laboratory.

‡ Numbers in parentheses, average MCR for this laboratory, expressed as liter/day per m^2.

TABLE III

Estrone-3-Sulfate-6,7-^3H Infusions

Subject	Sex and age	E_1S-^3H	Steroid	Plasma tritium levels								C E_1-^3H/E_1S-^3H E_2-^3H/E_1S-^3H	ρE_1SE_1*	ρE_1SE_2*
				1.75 hr	2.0 hr	2.25 hr	2.75 hr	4.0 hr	6.0 hr	7.0 hr	8.0 hr			
		cpm/day $\times 10^{-3}$		*cpm* $\times 10^{-3}$										
11	M 21	1.440	E_1S	387	427	441	519							
			E_1		3.30	3.64	4.523							
			E_1/E_1S		0.0074	0.0083	0.0087					0.0081	0.127	
			E_2		0.422	0.467	0.521							
			E_2/E_1S		0.00099	0.0011	0.0010					0.0010		0.10
12	F 19	1.203	E_1S	337	374	394	436							
			E_1	3.97	4.65	4.67	5.40							
			E_1/E_1S	0.012	0.012	0.012	0.012					0.012	0.180	
			E_2	0.661	0.725	0.636	0.596							
			E_2/E_1S	0.0020	0.0019	0.0016	0.0014					0.0017		0.012
13	F 20	0.9916	E_1S	266	266	278	277							
			E_1	5.61	5.75	5.90	5.98							
			E_1/E_1S	0.021	0.022	0.021	0.022					0.021	0.30	
			E_2	0.782	0.794	0.756	0.751							
			E_2/E_1S	0.0029	0.0030	0.0027	0.0027					0.0028		0.019
14	F 19	1.100	E_1S			317	333	344	392	506				
			E_1			6.02	6.98	7.62	7.84	10.7				
			E_1/E_1S			0.0190	0.021	0.022	0.020	0.021		0.021	0.231	
			E_2			—	—	—	—	—		—		—
			E_2/E_1S			—	—	—	—	—				
6‡	F 18	0.5250	E_1S		267			247	289	265	288			
			E_1		5.93			5.71	7.59	7.08	7.25			
			E_1/E_1S		0.022			0.023	0.026	0.027	0.025	0.025	0.19	
			E_2		0.966			0.935	0.972	1.01	0.985			
			E_2/E_1S		0.0036			0.0038	0.0034	0.0038	0.0034	0.0036		0.014
Mean													0.21	0.014

* Values for MCR_{E_1} or MCR_{E_2} performed in the same patient (Table V and VI) used for calculation of ρ. Otherwise average values from our laboratory used (Table III).

‡ Equilibrium was attained during infusion. MCR = 194 liter/day or 132 liter/day per m^2.

TABLE IV
Estrone-6,7-

Subject	Sex and age	Estrone-^{3}H infusion	^{3}H-steroid	Plasma tritium levels 1.75 hr	2.0 hr	2.25 hr
		cpm/day × 10^{-3}			*cpm* × 10^{-3}/*liter*	
[11]	M 21	1.048	E_1	41.7	44.6	47.2
			E_1S	86.5	99.4	110
12	F 19	0.965	E_1	45.4	43.5	43.5
			E_1S	89.3	101	117
13	F 20	0.992	E_1	43.8	45.2	49.0
			E_1S	60.6	67.0	75.6
8	F 18	1.08	E_1		(59.2)	
			E_1S		222	
Mean						

* Value for MCR_{E_1S} performed in the same patient (Table II) used for calculation of ρ; otherwise average values for our laboratory used (Table III).

time. The average MCR_{E_1S} in five men was 167 liter/day or 87.4 liter/day per m^2. The average MCR of five women was 146 liter/day or 94.1 liter/day per m^2. The large differences among normal subjects are related both to differences in fractional turnover rate, γ (range 0.0679–0.219 pools/hr) and volume of distribution, V (range 20–93 liters). The range of volumes of distribution was roughly correlated with body surface area.

The validity of calculation of MCR_{E_1S} based on the assumption of a one-compartment system was tested by comparing in the same subject the MCR obtained in this way with that obtained by the constant infusion technique. In this study, we injected a bolus of E_1S into a normal subject. No. 6, and established a MCR_{E_1S} from the plasma disappearance curve over 8 hr. Subsequently, a calculated bolus of E_1S-^{3}H was given followed by a continuous infusion of E_1S-^{3}H for a further 8 hr achieving a constant plasma level of E_1S-^{3}H (Fig. 3). From the data obtained after the injection of E_1S-^{3}H (Table I), a MCR of 207 liter/day was calculated. The MCR calculated from the constant infusion data (Table III) was 194 liter/day. These two MCR are not different.

The production rate of E_1S was calculated from the MCR_{E_1S} and the measured plasma concentrations of E_1S (Table II). Average values for MCR and plasma E_1S concentrations in both men and women were used. In men, E_1S production averaged 77 μg/day, not greatly different from E_1 and E_2 production rates. In women, E_1S production averaged 95 μg/day during the early follicular phase; and 182 μg/day during the luteal phase. Follicular and luteal phase E_1S production rates are compared with follicular and luteal E_1 and E_2 production rates (Table II). To convert E_1S production rates to equivalent E_1 production rates, multiply E_1S values by 0.71.

Conversion ratios. $C^{E_1 \leftarrow E_1}$ and $C^{E_1 \leftarrow E_2}$ were measured in five subjects (Table III). Shown in Table III are the plasma levels of tritium in E_1,E_2, and E_1S; conversion ratios have been calculated at each time point. Though plasma levels of E_1S-^{3}H, E_1-^{3}H, and E_2-^{3}H are increasing, the conversion ratios are constant and appear to represent equilibrium values. Each series of conversion ratios was examined for systematic trends or deviations from the mean. Only in subject 11 was there a suggestion that $C^{E_1 \leftarrow E_1}$ was increasing during the period of observation. In subject 6, plasma levels of E_1-^{3}H and E_1S-^{3}H reached equilibrium as did the conversion ratios and the conversion ratio of 0.025, was the same as the average of the other subjects. The conversion ratio ($C^{E_1 \leftarrow E_1}$) averaged 0.017 in five studies. Transfer factors (ρ) were calculated using these conversion ratios and values for MCR_{E_1} and MCR_{E_1S} either measured in the same subject (Table IV) or using average values from our laboratory (Table III) (3). Transfer factor ($\rho^{E_1 \leftarrow E_1}$) averaged 0.21 (range 0.13–0.30) (Table III).

The conversion ratio ($C^{E_1 \leftarrow E_2}$) averaged 0.0023 in four studies, about 1/10 that for the conversion of E_1S to estrone. Transfer factors were then calculated using these conversion ratios and values for MCR_{E_2} and MCR_{E_1S} either measured in the same subject (Table V) or using average values from our laboratory (Table II) (3). The transfer factor ($\rho^{E_1 \leftarrow E_2}$) averaged 0.014 (range 0.010–0.019) (Table III).

Since E_1S is effective by mouth in contrast to estrone and estradiol, the metabolism of E_1S taken orally to

^3H *Infusions*

Plasma tritium levels			Equilibrium	CE_1E_1S			
2.75 hr	3.0 hr	3.5 hr	^3H levels	E_1S-^3H/E_1-^3H	MCR_{E_1}	MCR_{E_1}	ρE_1E_1S*
cpm × 10^{-3}/*liter*			*cpm/liter* × 10^{-3}		*liter/day*	*liter/day/m²*	
49.2			45.7	10.3	2290	1350	0.67
123			471				
45.0			44.3	8.68	2180	1400	0.58
142			385				
47.4			46.3	5.34	2140	1310	0.38
83.2			247				
	66.7	67.6	67.2	12.4	1610	1020	0.54
	288	335	833				
				9.18			0.54

plasma E_1S, estrone, and estradiol was studied. When 30 μCi of E_1S-^3H was given by mouth. there was a prompt rise in plasma levels of tritiated E_1S, peaking around 1 hr as shown in Fig. 4. Plasma levels of tritiated E_1 and E_2 were 1.0% and 0.1%, respectively, that of E_1S-^3H, although E_2-^3H was detectable only at 80 min. These values are in close agreement with the conversion ratios found during i.v. infusions of tritiated E_1S shown in Table III.

Conversion of E_1 to E_1S. The data from the estrone-^3H infusions are shown in Table IV. Plasma level of the infused estrone became constant in all cases. However, conversion of estrone to E_1S did not reach equilibrium in any infusion, as shown by increasing plasma levels of E_1S-^3H. Thus the conversion ratio, could not be calculated directly since equilibrium conditions were not met. Estimates of equilibrium values for estrone-3-sulfate and hence conversion ratios, $C^{E_1E_1S}$, were calculated using the formula,

$$C^{E_1E_1S} = \frac{E_1S_{tx} - {}^3H}{(E_1 - {}^3H)(1 - e^{-\gamma T_x})}, \quad \text{(see Appendix)}$$

where E_1-^3H is the equilibrium value for E_1-^3H during E_1-^3H infusions and $E_1S_{t_x}$-^3H is the plasma level of tritium in E_1S at time T_x; e is the natural logarithm, T_x is the time after beginning the infusion; and γ is the fractional pool turnover of E_1S per unit time. In subject 8, γ was measured directly and average values for γ from Table II were used for subjects 11–13. In one subject, No. 18, in whom MCR_{E_1}, MCR_{E_1S}, and plasma levels of all estrogens had been measured, the SAAM program of Berman and Weiss (12) was used to perform a computer simulation and calculate equilibrium values for E_1S during a short E_1 infusion. There was agreement between this value and that obtained by the extrapolation shown above.

The average of four determinations of $C^{E_1E_1S}$ in plasma was 9.2 (range 5.34–12.4) (Table IV). Transfer factors were calculated using the conversion ratios, measured values for MCR_{E_1}, and average male and female MCR_{E_1S} from Table I (an MCR_{E_1S} was measured

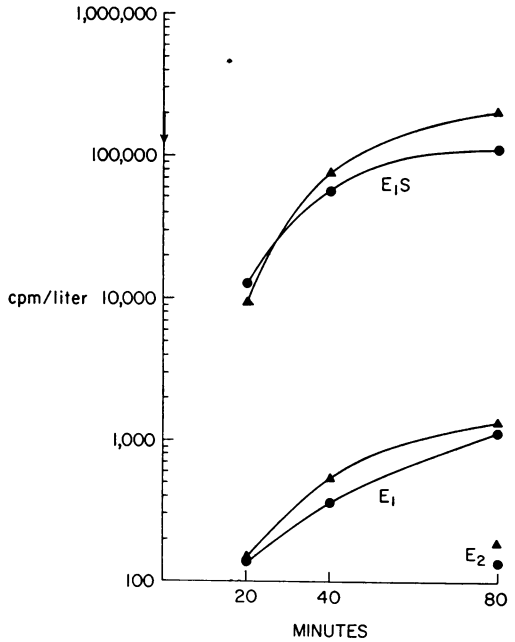

FIGURE 4 Plasma levels of tritium in E_1S; estrone (E_1), and estradiol (E_2) after oral administration of E_1S-^3H at time 0 (subjects 1 and 2).

TABLE V
Estradiol-6, 7-

Subject	Sex and age	Estradiol-^{3}H infusion	^{3}H-steroid	Plasma tritium levels 1.75 hr	2.0 hr	2.25 hr	2.75 hr
		cpm/day × 10^{-6}			*cpm* × 10^{-3}/*liter*		
7	F 18	1.32	E_2		111		
			E_1S		206		
9	F 22	0.919	E_2				
			E_1S				
15	F 22	1.27	E_2	140	129	136	148
			E_1S	106	111	122	154
16	M 26	1.14	E_2	69.3	73.2	72.1	
			E_1S	26.7	—	38.9	
17	M 26	1.06	E_2	58.1	58.9	62.6	
			E_1S	135	167	211	
18	M 23	1.26	E_2	60.1	60.9	64.9	
			E_1S	78.4	79.2	100	
Mean							

* Value for CMR_{ES_1} performed in the same patient (Table II) used for calculation of ρ; otherwise average values for our laboratory used (Table III).

and used for calculations in subject 8). The transfer factor ($\rho^{E_1E_1S}$) averaged 0.54 (Table IV).

Conversion of E_2 to E_1S. The data obtained from estradiol-6,7-^{3}H infusions are shown in Table V. Plasma levels of the infused steroid, estradiol, were at equilibrium in all cases, but as with E_1 the conversion of E_2 to E_1S did not reach equilibrium. The extrapolation to equilibrium values was performed as for E_1. Again, the SAAM program of Berman and Weiss (12) was used to confirm the validity of the estimates of equilibrium values calculated by the above formula. This was done in subject 7 in whom we had sufficient data about MCR and plasma estrogen concentrations to allow the computer to derive equilibrium solutions. There was good agreement between the two methods.

The average of six determinations of $C^{E_2E_1S}$ is 7.1; range 2.19–13.6) (Table V). Transfer factors were calculated using these conversion ratios, actual measured values for MCR_{E_2}, and average male and female values for MCR_{E_1S} from Table I (MCR_{E_1S} was measured and used in the calculation of transfer factor in subjects 7 and 9). The transfer factor ($\rho^{E_2E_1S}$) averaged 0.65 (Table V).

DISCUSSION

Estrone sulfate was identified in urine over 30 years ago but little is known of its role in the estrogen economy of the body. Although urinary estrogens are generally excreted as the glucuronide conjugates, in an occasional subject (13, 14). E_1S has been the major urinary estrogenic metabolite. As Purdy et al. (2) showed, it is also the major plasma metabolite of estradiol. We had postulated that there was a large plasma compartment that slowly exchanged with plasma estrone and estradiol (3), and it seemed likely that this was E_1S. This and the lack of information concerning origin, clearance rates, and metabolism of E_1S prompted the studies reported here.

The MCR_{E_1S} was obtained by fitting the data obtained after rapid injection of E_1S-^{3}H to a straight line thereby assuming a one-compartment model. Frequent samples were not obtained during the first hr so that a rapid component of the disappearance curve would have been missed. In two studies, the 30 min sample suggested an early faster component but this could exert little effect on the calculation of the MCR since the total rate of removal of E_1S from plasma is so slow. The study in subject 6 directly compared the MCR obtained assuming distribution into a single compartment and that obtained during constant infusion when plasma concentrations of E_1S-^{3}H were at equilibrium. Since results obtained by the latter method are independent of the compartmental distribution of the isotope, the agreement between these data is validation of our measurement of MCR_{E_1S}. In the single study of the clearance of DHS, a one-compartment model also was adequate to explain the distribution of the steroid (15). The estimated MCR_{E_1S} of 150 liter/day is in agreement with

^3H Infusions

Plasma tritium levels				Equilibrium	$C_{E_2E_1S}$			
3.0 hr	3.5 hr	5.0 hr	7.0 hr	^3H levels	E_1S-^3H/E_2-^3H	MCR_{E_2}	MCR_{E_2}	$\rho_{E_2E_1S}$*
	cpm × 10^{-3}/*liter*			*cpm/liter* × 10^{-3}		*liter/day*	*liter/day/m²*	
111	108			110	11.0	1200	739	0.59
293	318			1210				
58		68	81	74.5	5.8	1230	832	0.81
108		200	275	432				
				138	3.08	920	520	0.56
				425				
				71.5	2.19	1594	886	0.22
				157				
				59.9	13.6	1770	983	1.20
				815				
				62.0	6.68	2032	1129	0.52
				414				
					[7.06]			0.65

data reported recently by Longcope (16) and confirms the early work of Twombly and Levitz (1) who showed that E_1S is cleared from the blood slowly.

The plasma MCR_{E_1S} of 150 liter/day is considerably higher than the plasma MCR of DHS (7 liter/day) (15), and of the blood MCR of DHS (16–30 liter/day) (17), testosterone sulfate (25 liter/day) (17), 17-acetoxypregnenolone sulfate (37 liter/day) (18). A recent report gives values for testosterone sulfate MCR of 75–240 liter/day which are in the same range as our estimates of MCR_{E_1S} (19). However, the fractional pool turnover rate of E_1S of 3.3 pools/day is about the same as the fractional pool turnover rates of other steroid sulfates (15, 17–19). Thus, the higher MCR_{E_1S} must be associated with a larger volume of distribution of E_1S, and in fact its volume of distribution of about 50 liters is higher than those of the other steroids (15, 17, 18) which have lower MCR. Only pregnenolone sulfate had a higher MCR than E_1S but it had a relatively small volume of distribution (7 liters) and a higher fractional turnover rate almost equal to that of some unconjugated steroids (18).

The volume of distribution of the steroid sulfate depends on the binding of the sulfates to plasma proteins, in particular to albumin. Binding to albumin in man has been demonstrated by Puche and Nes (20), Sandberg and Slaunwhite (21), and Plager (22). Puche and Nes, Plager, and Wang and Bulbrook (23) noted that all steroid sulfates tested competed for the same binding sites. However, the association constants were influenced by the nature of the steroid. The association constant of DHS with albumin has been reported as $0.6–2 \times 10^5$ liter/mole (20, 22), whereas the E_1S binding constant was estimated at 2×10^4 liter/mole (24). Although these binding affinities were not obtained in the same system, the association of lower affinity constants with higher volumes of distribution is apparent. It therefore seems probable that the clearance rates of the steroid sulfates vary inversely with the tightness of binding to albumin which in turn determines the volume of distribution.

Using the average MCR_{E_1S} and mean E_1S plasma concentrations, we found that plasma production rates of E_1S were similar to those of estrone and estradiol in men and women. Although we have not looked for possible variations of E_1S concentrations throughout the day, it can be shown that they will not vary greatly because of the low fractional pool turnover rate (γ). Thus, the E_1S production rates need not be corrected for a mean plasma E_1S concentration, and should closely approximate the 24-hr production rates of E_1S.

Since sulfokinases are present in peripheral tissues as well as in the gonads and adrenal cortex, we could not know to what extent E_1S was either secreted[2] or

[2] The term "secretion" is used to indicate entry of the steroid into the plasma from the glands. The term "production" signifies the rate of entry of the steroid from all sources.

produced from peripheral transformation of plasma precursors. We therefore calculated the contributions of plasma estrone and estradiol to the E_1S production rate. To do this, we used our values for transfer factors, $\rho^{E_1E_1S}$, $\rho^{E_2E_1S}$; $\rho^{E_1SE_1}$, $\rho^{E_1SE_2}$ (Tables II–V) as well as the average transfer factors, $\rho^{E_1E_2}$, $\rho^{E_2E_1}$ (25, 26). The following three equations give solutions for the net amount of steroid entering the plasma from all sources (shown by [ ]) other than from conversion of the two other steroids:

$$[E_1] = PR_{E_1} - \rho^{E_1SE_1}[E_1S] - \rho^{E_2E_1}[E_2],$$
$$[E_2] = PR_{E_2} - \rho^{E_1SE_2}[E_1S] - \rho^{E_1E_2}[E_1],$$
$$[E_1S] = PR_{E_1S} - \rho^{E_1E_1S}[E_1] - \rho^{E_2E_1S}[E_2].$$

Since the production rates (PR) and transfer factors are known, the equations can be solved for the [ ] values. These values are not true secretion rates since they would include any steroid produced from other precursors. For example, $[E_1]$ would include that estrone derived from plasma androstenedione and testosterone as well as that moiety that is secreted.

The derived data are given in Table VI. For each subgroup, $[E_1S]$ was negative. This means that the production rate calculated to result from conversion of plasma estrone and estradiol is higher than that calculated by direct measurement of the MCR_{E_1S} and plasma E_1S concentration. Hence, there is no need to postulate glandular secretion of E_1S. The lack of correspondence of the data must be explained and serves to point out the many sources of discrepancies in all studies such as this.

The first and most important reason for lack of correspondence in calculations such as these is not really "error" but relates to the large range of normal average values for metabolic clearance rates and transfer factors that are used. Inspection of published data for metabolic clearance rates of E_1 and E_2 (3, 25, 26) reveals not only a large range of values for normals but also for groups of normals studied in the same laboratory (25, 26). This same large range of normal values is also evident from our data for $\rho^{E_1E_1S}$, $\rho^{E_2E_1S}$, and MCR_{E_1S}. Since our study included only a small sample from a population with a large normal range, it is possible that our average values for $\rho^{E_1E_1S}$, $\rho^{E_2E_1S}$ could be considerably higher than the true mean for the normal population. Agreement between the calculations would be possible only if all parameters were measured in the same normal subjects. Secondly, average values for estradiol concentrations in men reported by us previously (5) and used for our calculations of E_2 production rates are somewhat higher than more recent values of 20–25 pg/ml reported by other laboratories (9–11). The errors in measurement of low estrogen concentrations by radioligand assays tend to cause high values which would result in overestimation of production rates. The consistent finding that estrogen production rates, calculated from dilution of labeled estrogen into urinary metabolites, are smaller than those calculated from the MCR and plasma concentration is further evidence that the latter method overestimates the production rate. Next, the known diurnal variations in plasma estrone concentration (27, 28) and possibly of estradiol, mean that production rates obtained using morning plasma estrogen concentrations will systematically overestimate the 24 hr estrogen production rate of these unconjugated estrogens. Finally, since plasma estradiol and estrone have plasma turnovers of about 50 pools/day and E_1S turnover rate is 3.3 pools/day, plasma concentrations of E_1S will lag behind plasma estrone and estradiol by 6–12 hr. Therefore, it will be difficult to arrive at correct sampling times to estimate the contributions of estrone and estradiol to E_1S production rates.

Since most of the errors discussed will cause overestimation of estrone and estradiol blood production rates, the amount of E_1S calculated to arise from these sources will also be overestimated. Nevertheless, all E_1S found in plasma can be accounted for merely by using mean values ± standard deviations for production rates and ρ used in the calculations. Hence, it is untenable

TABLE VI
Origin of Estrone Sulfate

	$[E_1]$*	$\rho E_1E_1S[E_1]$	$[E_2]$	$\rho E_2E_1S[E_2]$	$[E_1S]$	PR E_1S (from E_1 and E_2)	PR E_1S (MCR × i)
Men	153‡	82.6	74.5	48.5	−54	131	77
Women Follicular phase	137	74	157	102	−81	176	95
Women Luteal phase	175	94.5	279	181	−94	275	182

* [ ] = the amount of steroid entering the plasma from all sources other than the two other steroids.
‡ All values are micrograms per day, expressed as E_1S.

to suggest (29) that E_1S may be secreted in amounts sufficient to make it a significant precursor of plasma estrone.

The transfer factor, $\rho^{E_1SE_1}$ of 0.21 was derived from the conversion ratios obtained from the short infusions of E_1S during which plasma levels of E_1S-^3H, E_1-^3H, and E_2-^3H were increasing in four of the five studies. However, $\rho^{E_1SE_1}$ and $\rho^{E_1SE_2}$ appeared to be at equilibrium. The $\rho^{E_1SE_1}$ of 0.21 agrees closely to that of 0.15 reported recently by Longcope (16). The transfer factor, $\rho^{E_1SE_2}$ was only 10% of that for estrone (Table IV). Since $\rho^{E_1E_2}$ is also about 10% (25, 26), it is probable that E_1S reaches the plasma estradiol compartment via plasma estrone. Although the mass of estrone sulfate available for exchange with E_1 is large, about 25–50 μg (plasma concentration × volume of distribution), only 5–10 μg could enter the plasma volume daily as estrone. This is a small fraction of estimated estrone production rates of 40–200 μg/day in men and women.

Although $\rho^{E_1SE_1}$ and $\rho^{E_1SE_2}$ were apparently at equilibrium, computer simulations of these studies[3] using the SAAM program of Berman and Weiss (12) have shown that these conversion ratios cannot be at equilibrium and that the true conversion ratio may be greater by about 15%. Short sampling times and variability in the determinations mask this trend. For the purposes of understanding these facets of estrone sulfate metabolism, the values used in our calculations are sufficiently accurate although they are not equilibrium values.

In contrast to the low biologic potency of orally administered estrone and estradiol, E_1S is an active oral estrogen. Our data confirm that it is rapidly absorbed from the gut and reaches the peripheral circulation without significant metabolism by the liver. Since 0.5 mg of estrone sulfate is an adequate replacement dose for many postmenapausal women, we have calculated that the plasma estrone production rate resulting from this dose is 70 μg/day ($0.5 \times 0.7 \times 0.2$).[4] Similarly, the resultant estradiol production rate would be 7 μg/day. The minimal estrogen production rates that cause either vaginal cornification or endometrial hyperplasia are unknown but Grodin and McDonald[5] found that an estrone production rate greater than 40 μg/day (by urinary isotope dilution) induced endometrial hyperplasia in women. The estrone production rate of normal postmenapausal women calculated from plasma concentrations and metabolic clearance rates is about 40 μg/day (30) and there is little biologic estrogenic effect at these levels. Thus it may be that the conversion of orally administered estrone sulfate to plasma estrone and estradiol is sufficient to account for its biologic effect. In this regard, it is of interest that Gurpide, Stolee, and Tseng (31) reported that human endomentrium rapidly metabolized estrone sulfate to estrone and estradiol.

In summary: (*a*) E_1S is a major circulating plasma estrogen and has a long plasma half-life; (*b*) the large contributions of estrone and estradiol to plasma E_1S are more than sufficient to account for all the circulating plasma E_1S; (*c*) orally administered E_1S is metabolized to plasma estrone and estradiol to the same extent as i.v. administered E_1S.

APPENDIX

Because of the slow fractional turnover rate of E_1S, measurement of $\rho^{E_1E_1S}$ and $\rho^{E_2E_1S}$ at equilibrium values of E_1, E_2, and E_1S was impossible unless infusions were carried out for longer than 24 hr. Since the dissappearance of E_1S from plasma can be adequately described by a single exponential, this same single exponential describes the appearance of E_1S in plasma during an infusion of $E_1 - {}^3H$ or $E_2 - {}^3H$. When plasma levels of $E_1 - {}^3H$ and $E_2 - {}^3H$ are at equilibrium, a constant amount of $E_1 - {}^3H$ or $E_2 - {}^3H$ is being metabolized to E_1S. Thus when estrone or estradiol is infused at rates such that their plasma levels reach equilibrium values, E_1S enters the plasma at a rate equal to the $\rho^{E_1E_1S}$ or $\rho^{E_2E_1S}$ × the infusion rate of $E_1 - {}^3H$ or $E_2 - {}^3H$. We were therefore able to estimate $C^{E_1E_1S}$, $C^{E_2E_1S}$ during short infusions of either $E_1 - {}^3H$ or $E_2 - {}^3H$ by making the following assumptions:

(*a*) The bolus given before the E_1 or E_2 infusions adequately sets the plasma $E_1 - {}^3H$ or $E_2 - {}^3H$ at an equilibrium level that is then maintained by the constant $E_1 - {}^3H$ or $E_2 - {}^3H$ infusions. (*b*) A constant amount of $E_1 - {}^3H$ or $E_2 - {}^3H$ is being metabolized to $E_1S - {}^3H$ from the time 0 when the bolus is given and hence a constant amount of $E_1S - {}^3H$ is entering the plasma E_1S pool during the entire time of the short E_1 or E_2 infusion. To verify these assumptions, we performed computer simulations of typical $E_1 - {}^3H$ or $E_2 - {}^3H$ infusions utilizing the computer model program of Berman and Weiss (12). We were able to show that the dose of $E_1 - {}^3H$ or $E_2 - {}^3H$ given as a bolus to begin the short infusions adequately set the plasma level of $E_1 - {}^3H$ or $E_2 - {}^3H$ at near equilibrium levels; and that in addition, a constant amount of $E_1S - {}^3H$ is entering the plasma E_1S pool from the time of the bolus at time 0.

Thus, the instantaneous change in $E_1S - {}^3H$ is described by the following,

$$\frac{dE_1S_{t_x}}{dt} = k_1E_{1t_x} - \gamma E_1S_{t_x},$$

where $E_1S_{t_x}$ is the instantaneous level of $E_1S - {}^3H$ at time t_x; E_1 is the instantaneous level of $E_1 - {}^3H$ at time t_x; k_1 is the fractional turnover of $E_1 - {}^3H$ to $E_1S - {}^3H$; and γ is the total rate of removal of $E_1S - {}^3H$ as given in Table I. Integrating and solving for $E_1S_{t_x}$ yields,

$$E_1S_{t_x} = \frac{k_1E_{1t_x}}{\gamma}(1 - e^{-\gamma T_x}),$$

where e is natural logarithm; T_x is time after beginning the studies. By definition, however $C^{E_1E_1S} = E_1S - {}^3H/E_1 - {}^3H$

[3] Ruder, H. J., L. Loriaux, M. B. Lipsett, and M. Berman. Manuscript in preparation.

[4] Dose of E_1S is 0.5 mg/day; the transfer constant, $\rho^{E_1SE_1}$ is 0.2 and only 70% of E_1S is estrone by weight.

[5] Grodin and McDonald. Personal communication.

when both are equilibrium values. And in fact, $E_{1t_x} - {}^3H$ is at equilibrium from time 0 as explained above and will be written $E_1 - {}^3H$. Hence, $E_{1t_x} - {}^3H = E_1 - {}^3H$. At time $T_x = \infty$, E_1S is also at equilibrium by defintion and $e^{-\gamma T_x} = 0$ and yields:

$$E_1S_\infty = \frac{k_1E_1}{\gamma}(1 - 0).$$

Rearranging yields:

$$\frac{E_1S}{E_1} = \frac{k_1}{\gamma}.$$

Since $C^{E_1E_1S} = E_1S - {}^3H/E_1 - {}^3H$; $C^{E_1E_1S} = k_1/\gamma$. Thus,

$$E_1S_{t_x} - {}^3H = C^{E_1E_1S} \cdot (E_1 - {}^3H)(1 - e^{-\gamma T_x}).$$

And rearranging:

$$C^{E_1E_1S} = \frac{E_1S_{t_x} - {}^3H}{(E_1 - {}^3H)(1 - e^{-\gamma T_x})}$$

The same formula holds for $C^{E_2E_1S}$ substituting appropriate values for E_2 and γ. All conversion ratios shown in Tables IV and V were calculated using this formula and, where possible, the actual measured value for γ. Otherwise, average values for γ were used from Table I. Since each infusion had at least three plasma samples at different time points, conversion ratios were calculated at each time point during a single infusion and averaged, yielding the values shown in Tables IV and V. Validity of these calculations was confirmed by using data from subjects 7 and 8 in whom we had estimates of all MCR and plasma concentrations for computer simulation using the SAAM program of Berman and Weiss (12). There was close agreement between conversion ratios predicted by the computer program and the conversion ratios calculated by the above formula.

ACKNOWLEDGMENTS

The assistance of Dr. Monas Berman in teaching the authors the use of the SAAM computer program and of Dr. David Rodbard for his aid in the derivation of the mathematical formulas is gratefully acknowledged.

REFERENCES

1. Twombly, G. H., and M. Levitz. 1960. Metabolism of estrone-C^{14}-16 sulfate in women. *Amer. J. Obstet. Gynecol.* **80:** 889.
2. Purdy, R. H., L. L. Engel, and J. L. Oncley. 1961. The characterization of estrone sulfate from human plasma. *J. Biol. Chem.* **236:** 1043.
3. Hembree, W. C., C. W. Bardin, and M. B. Lipsett. 1969. A study of estrogen metabolic clearance rates and transfer rates. *J. Clin. Invest.* **48:** 1809.
4. Mumma, R. O., C. P. Hoiberg, and W. W. Weber. 1969. Preparation of sulfate esters. The synthesis of steroid sulfates by a dicyclohexyl-carbodiimide medicated sulfation. *Steroids.* **14:** 67.
5. Loriaux, D. L., H. J. Ruder, and M. B. Lipsett. 1971. The measurement of estrone sulfate in plasma. *Steroids.* **18:** 463.
6. Ruder, H. J., P. Corvol, J. A. Mahoudeau, G. T. Ross, and M. B. Lipsett. 1971. Effects of induced hyperthyroidism on steroid metabolism in men. *J. Clin. Endocrinol. Metab.* **33:** 382.
7. Bardin, C. W., and M. B. Lipsett. 1967. Testosterone and androstenedione blood production rates in normal women with idiopathic hirsutism or polycystic ovaries. *J. Clin. Invest.* **46:** 891.
8. Horton, R., and J. F. Tait. 1966. Androstenedione production and interconversion rates measured in peripheral blood and studies on the possible site of its conversion to testosterone. *J. Clin. Invest.* **45:** 301.
9. Baird, D. T. 1968. A method for the measurement of estrone and estradiol-17 in peripheral human blood and other biological fluids using ^{35}S pipsyl chloride. *J. Clin. Endocrinol. Metab.* **28:** 244.
10. Korenman, S. G., L. E. Perrin, and T. P. McCallum. 1969. A radio-ligand binding assay system for estradiol measurement in human plasma. *J. Clin. Endocrinol. Metab.* **29:** 879.
11. Nagai, N., and C. Longcope. 1971. Estradiol-17 and estrone: studies on their binding to rabbit uterine cytosol and their concentration in plasma. *Steroids.* **17:** 631.
12. Berman, M., and M. F. Weiss. 1967. Users manual for SAAM. U. S. Government Printing Office, Washington, D. C. Publication No. 1703.
13. Howard, G. M., H. Robinson, F. H. Schmidt, J. R. Mccord, and J. R. Preedy. 1969. Evidence for a two-pool system governing the excretion of radioactive urinary estrogen conjugates during the first eight hours following the administration of estrone-6,7-^3H to male subjects. Probable role of the enterohepatic circulation. *J. Clin. Endocrinol. Metab.* **29:** 1618.
14. Hobkirk, R., M. Nilsen, and P. R. Blahey. 1969. Conjugation of urinary phenolic steroids in the nonpregnant human female with particular reference to estrone sulfate. *J. Clin. Endocrinol. Metab.* **29:** 328.
15. Sandberg, E., E. Gurpide, and S. Lieberman. 1964. Quantitative studies on the metabolism of dehydroisoandrosterone sulfate. *Biochemistry.* **3:** 1256.
16. Longcope, C. 1972. The metabolism of estrone sulfate in normal males. *J. Clin. Endocrinol. Metab.* **34:** 113.
17. Wang, D. Y., R. D. Bulbrook, A. Sneddon, and T. Hamilton. 1967. The metabolic clearance rates of dehydroepiandrosterone, testosterone and their sulfate esters in man, rat and rabbit. *J. Endocrinol.* **38:** 307.
18. Wang, D. Y., D. R. Bulbrook, F. Ellis, and M. M. Coombs. 1967. Metabolic clearance rates of pregnenolone, 17-acetoxypregnenolone and their sulphate esters in man and in rabbit. *J. Endocrinol.* **39:** 395.
19. Saez, J. M., J. Bertrand, and C. J. Migeon. 1971. Metabolic clearance rate, urinary and plasma production rates of testosterone sulfate in man. *Steroids.* **17:** 435.
20. Puche, R. C., and W. R. Nes. 1962. Binding of dehydroepiandrosterone sulfate to serum albumin. *Endocrinology.* **70:** 857.
21. Sandberg, A. A., and W. R. Slaunwhite. 1957. The binding of steroids and steroid conjugates to human plasma proteins. *Recent Progr. Hormone Res.* **13:** 209.
22. Plager, J. E. 1965. The binding of androsterone sulfate, etiocholanolone sulfate, and dehydroisoandrosterone sulfate by human plasma protein. *J. Clin. Invest.* **44:** 1234.
23. Wang, D. Y., and R. D. Bulbrook. 1967. Binding of the sulfate esters of dehydroepiandrosterone, testosterone, 17-acetoxypregnenolone and pregnenolone in the plasma of man, rabbit and rat. *J. Endocrinol.* **39:** 405.
24. Sandberg, A. A., H. Rosenthal, S. L. Schneider, and W. R. Slaunwhite. 1966. Steroid interactions and their

role in the transport of steroids. *In* Steroid Dynamics. G. Pincus, T. Nokao, and J. F. Tait, editors. Academic Press, Inc., New York. 1–61.
25. Longcope, C., D. S. Layne, and J. F. Tait. 1968. Metabolic clearance rates and interconversions of estrone and 17-estradiol in normal males and females. *J. Clin. Invest.* **47**: 93.
26. Longcope, C., and J. F. Tait. 1971. Validity of metabolic clearance and interconversion rates of estrone and 17-estradiol in normal adults. *J. Clin. Endocrinol. Metab.* **32**: 481.
27. Baird, D. T., and A. Guevara. 1969. Concentration of unconjugated estrone and estradiol in peripheral plasma in nonpregnant women throughout the menstrual cycle, castrate and postmenopausal women and in men. *J. Clin. Endocrinol. Metab.* **29**: 149.
28. Tulchinsky, D., and S. G. Korenman. 1970. A radioligand assay for plasma estrone; normal values and variation during the menstrual cycle. *J. Clin. Endocrinol. Metab.* **31**: 76.
29. Baird, D., R. Horton, C. Longcope, and J. F. Tait. 1968. Steroid prehormones. *Perspect. Biol. Med.* **11**: 384.
30. Longcope, C. 1971. Metabolic clearance and blood production rates of estrogens in postmenopausal women. *Amer. J. Obstet. Gynecol.* **111**: 778.
31. Gurpide, E., A. Stolee, and L. Tseng. 1971. Quantitative studies of tissue uptake and disposition of hormones. *Acta Endocrinol. Suppl.* 153.